VOL. XXXIII. MAY, 1905. NO. 5.

AMERICAN

CHEMICAL JOURNAL

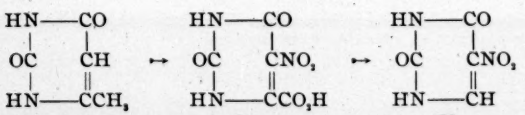
Contributions from the Sheffield Laboratory of Yale University.

CXXII. — RESEARCHES ON PYRIMIDINES: THE STRUCTURE OF SOME SUBSTITUTION-PRODUCTS.

[EIGHTH PAPER.]

BY HENRY L. WHEELER AND H. STANLEY BRISTOL.

When a pyrimidine derivative, having hydrogen both in the 4- and 5-positions, is attacked by the halogens or nitric acid, the question as to which hydrogen atom is replaced or substituted has not, hitherto, been decided. A method, which has now been successful in dealing with this question, depends on the structure of Behrend's nitro- and aminouracil. Nitrouracil has the nitro group in the 5-position, as it is prepared from 4-methyluracil,[1] (I.), or 5-nitrouracil-4-carboxylic acid[2], (II.):



I. II. III.

[1] Kohler: Ann. Chem. (Liebig), **236**, 50 (1886).
[2] Behrend: *Ibid.*, **229**, 36 (1885); **240**, 5 (1887).

Aminouracil results by reduction of the above nitrouracil, the amino group is, therefore, in the 5-position. Neither nitrouracil nor aminouracil has a definite melting or decomposing point. 5-Aminouracil picrate, however, has a definite effervescing point, which serves to identify it. Behrend and Grünwald[1] state that this compound melts at 147°–148°. This is incorrect, possibly a typographical error. It has been found, by several workers in this laboratory, that the action of nitric action on uracil, 2-ethylmercapto-6-oxypyrimidine, 2-thiouracil and other pyrimidine derivatives leads to the formation of a nitrouracil. In each case, after reduction with aluminium amalgam, the product gave a picrate effervescing sharply at 247°, or 100° higher than the temperature given by the above authors. At the request of one of us, Mr. C. O. Johns prepared 5-nitrouracil (III.) by Behrends' method and this, on reduction, gave a picrate showing no signs of melting at 147° but effervesced, as in the other cases, at 247°. The compounds were identical and it therefore follows that nitric acid attacks these various pyrimidine derivatives in the 5-position.

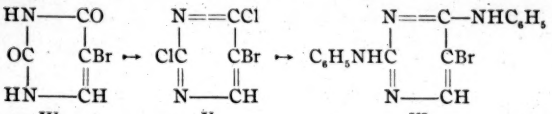
For the purpose of comparison, Traube's 2,6-dioxy-4-aminopyrimidine or 4-aminouracil[2] was prepared. When the saturated aqueous solution of this substance was mixed with a saturated solution of picric acid no precipitate resulted and no picrate was obtained.

It was stated in a previous article[3] that Dr. Merriam obtained 5-aminouracil when the product, obtained by brominating uracil, was heated with aqueous ammonia at 180°; it was identified by means of its picrate. A specimen of the picrate, prepared by Dr. Merriam, was found, by us, to sinter decidedly about 147°, but did not melt, or rather effervesce, until 247°. The compound proved to be identical with the above products and it therefore follows that bromine attacks uracil in the 5-position, as previously represented.

It might be expected that a 2-alkylmercapto-6-oxypyrimidine would also give a 5-brom derivative. This was proved to be the case as follows: 5-Bromuracil (IV.) was converted into

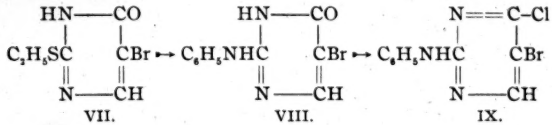
[1] Ann. Chem. (Liebig), **309**, 259 (1899).
[2] Ber. d. chem. Ges., **33**, 1381 (1900).
[3] THIS JOURNAL, **31**, 603 (1904).

2,6-dichlor-5-brompyrimidine (V.) by warming with a mixture of phosphorus oxychloride and phosphorus pentachloride and this, with aniline, gave 2,6-dianilino-5-brompyrimidine (VI.):



IV. V. VI.

2-Ethylmercapto-6-oxypyrimidine was treated with bromine, in glacial acetic acid and the resulting brom derivative (VII.) was warmed with aniline; this gave 2-anilino-5-brom-6-oxypyrimidine (VIII.), which was then converted into the chloride (IX.). When this chloride was warmed with aniline the product was found to be identical with the above dianilinobrompyrimidine (VI.), so that the process can be represented as follows:

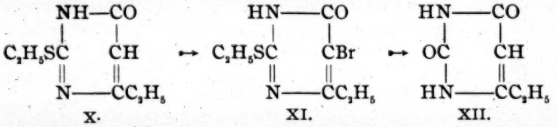


VII. VIII. IX.

Our first plan of procedure for deciding the question of the position of bromine in the mercaptooxypyrimidines, was to boil these products with hydrochloric acid and identify the bromuracil formed. It was found, however, that prolonged boiling of 2-ethylmercapto-5-brom-6-oxypyrimidine with hydrochloric acid gave uracil, the bromine being entirely removed. The 5-brom derivatives of uracil part with their halogen more readily than those of cytosine or, in other words, an oxygen in the 6-position renders bromine in the 5-position more active than when the 6-position is occupied by an amino group. This is true not only for the replacement of bromine by hydrogen, but also by the amino group.

We have found that 2-ethylmercapto-4-ethyl-5-brom-6-oxypyrimidine (XI.) also loses bromine by simply evaporating its solution, in concentrated hydrochloric acid, to dryness on the

water-bath, the product obtained being 4-ethyluracil (XII.). The 2-ethylmercapto-4-ethyl-6-oxypyrimidine (X.) for this experiment was prepared by the condensation of the ethyl bromide addition-product of thiourea and ethylpropionylacetate, $CH_3CH_2COCH_2CO.OC_2H_5$,[1] in alkaline solution.



X. XI. XII.

Bromine is represented in XI. as entering at the 5-position and not in the alkyl group. Behrend[2] has shown that when 4-methyluracil is brominated and then oxidized bromuracil-carboxylic acid results.

EXPERIMENTAL.

The uracil used in this work was prepared by boiling various 2-alkylmercapto-6-oxypyrimidines with concentrated hydrochloric acid. In the course of our experiments a number of new pseudoureas and alkylmercapto pyrimidines were employed. The former were prepared, for analysis, by treating pure, finely-powdered thiourea with a little alcohol and the alkyl halide was added. The mixtures were warmed on the steam-bath until solution took place and allowed to crystallize, in a vacuum, over sulphuric acid. The crystals were then simply washed with ether ; they were readily soluble in water and alcohol and insoluble in benzene and ether. It was found that pseudoethylthiourea hydrobromide dissolved in acetone, it was readily soluble in pyridine and it could be crystallized from acetic anhydride.

Pseudonormalpropylthiourea hydrobromide formed long, colorless needles, which melted at about 60°.

	Calculated for $C_4H_{14}N_2S.HBr$.	Found.
N	14.07	14.19

Pseudoisobutylthiourea hydrobromide formed a fibrous mass of crystals, melting at about 96°.

[1] Blaise : Compt. rend., **132**, 978 (1901).

[2] Ann. Chem. (Liebig), **240**, 22 (1887).

	Calculated for $C_5H_{13}N_2S.HBr$.	Found.
N	13.14	13.21

Pseudoisoamylthiourea hydrobromide crystallized in long prisms, melting at about 84°.

	Calculated for $C_4H_{14}N_2S.HBr$.	Found.
N	12.33	12.28

2-Normalpropylmercapto-6-oxypyrimidine, $C_3H_7SC_4H_2ON_2$ (HN——CO / N——CH, CH.—)

This and the following alkylmercaptooxypyrimidines were prepared by the method described in the case of the corresponding ethyl derivative.[1] The product from the condensation of 20 grams of the normalpropylbromide addition-product of thiourea, 30 grams of ethyl sodium formylacetate and 6 grams of sodium hydroxide, amounted to 12 grams. It was readily soluble in alcohol and hot water. From 40 per cent alcohol it formed long, colorless needles, melting at 117°.

	Calculated for $C_7H_{10}ON_2S$.	Found.
N	16.47	16.39

2-Isobutylmercapto-6-oxypyrimidine.—Twenty-one grams of the above pseudoisobutylthiourea hydrobromide, 38 grams of ethyl sodium formyl acetate and 4 grams of sodium hydroxide gave about 12 grams of this mercaptooxypyrimidine. When crystallized from 50 per cent alcohol it formed leaf-like plates, melting at 107°.

	Calculated for $C_8H_{12}ON_2S$.	Found.
N	15.21	15.19

2-Isoamylmercapto-6-oxypyrimidine crystallized from 50 per cent alcohol in long, thin plates, melting at 115°.

	Calculated for $C_9H_{14}ON_2S$.	Found.
N	14.14	14.08

Nitration of Uracil (By Mr. S. H. Clapp).—One gram of uracil was added to a mixture of 5 cc. of concentrated sul-

[1] THIS JOURNAL, **29**, 483 (1903).

phuric acid and 10 cc. of fuming nitric acid. The mixture was heated to about 125°, in an oil-bath, for an hour. On pouring into ice-water, nitrouracil separated in colorless, prismatic needles. The yield was about 1 gram. This compound was reduced by means of aluminium amalgam, in ammoniacal solution. On filtering and concentrating, aminouracil separated in balls of needles, which were recrystallized from water; they gave 33.47 per cent of nitrogen, the calculated being 33.07.

5-Aminouracil Picrate.—The above aminouracil was dissolved in warm water and a hot solution of picric acid was added, the picrate then separated in the form of yellow, rectangular plates which began to sinter strongly at 150°, then melted, with effervescence, at 247°–248°.

	Calculated for $C_{10}H_8O_9N_8$.	Found.
N	23.59	23.27

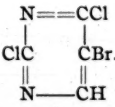
Nitration of 2-Ethylmercapto-6-oxypyrimidine.—2-Ethylmercapto-6-oxypyrimidine can be dissolved in a mixture of fuming nitric acid and concentrated sulphuric acid, at the ordinary temperature and be recovered, unaltered, on pouring into ice-water and neutralizing with sodium carbonate. If the mixture is warmed on the steam-bath until the reaction is well started, then allowed to stand in a warm place (about 60°), large crystals of nitrouracil separate.

The following was found to be the easiest method of preparing nitrouracil: Ten grams of 2-ethylmercapto-6-oxypyrimidine were dissolved in a mixture of 20 cc. of red, fuming nitric acid, 10 cc. of concentrated nitric acid and 10 cc. of concentrated sulphuric acid. The reaction was started by warming on the steam-bath, then the liquid was allowed to stand, at about 60°, for several hours, 4.9 grams of well-crystallized nitrouracil separated. The crystals were filtered and the acid filtrate allowed to stand for 24 hours longer, at 60°, whereupon 2.7 grams of nitrouracil separated, or 7.6 grams in all. This is a yield of 75.5 per cent of the calculated. When the compound was reduced by means of aluminium amalgam and the picrate prepared, it was found that the latter became

black at about 190°, but nevertheless effervesced sharply at 247°. The same partial decomposition and effervescing point was noticed in the case of the picrate prepared in the following experiment.

Nitration of 2-Thio-6-oxypyrimidine.—Twenty-five per cent nitric acid reacts energetically with this pyrimidine derivative and a portion is converted into nitrouracil. Eight grams of 2-thiouracil gave about 4 grams nitrouracil; beside this a far more soluble substance was formed, which gave a bulky precipitate with mercuric chloride, it was probably 6-oxypyrimidine, but was not isolated.

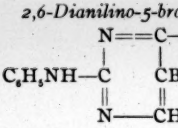
Nitration of 2-Ethylmercapto-6-chlorpyrimidine (By Dr. T. B. Johnson).—The mercaptochlorpyrimidine (1.5 grams) was added to a mixture of 4 cc. of concentrated nitric acid and 4 cc. of concentrated sulphuric acid. The temperature of the mixture was not allowed to rise above 90°; it was maintained at this point until effervescence ceased. The product was cooled, poured on to ice and crystallized from water; it contained 27.1 per cent nitrogen, the calculated percentage for nitrouracil is 26.7. This substance was reduced and the picrate prepared; it melted or effervesced at 247°.



2,6-Dichlor-5-brompyrimidine, —Eleven grams of 5-bromuracil, 13 grams of phosphorus pentachloride and 40 cc. of phosphorus oxychloride were mixed and warmed on the steam-bath. Hydrogen chloride was evolved and, after 4 days, all of the bromuracil dissolved. The solution was poured on to ice and extracted with ether. The ether was dried over calcium chloride and evaporated, leaving 8 grams of oil, which boiled at 119°–120° (17–18 mm.). It solidified, in a freezing-mixture, to a crystalline mass and melted at —3° – —2°.

	Calculated for $C_4HN_2Cl_2Br$.	Found.
N	12.28	12.46

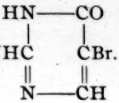
2,6-Dianilino-5-bromypyrimidine,



.—Six grams of 2,6-dichlor-5-brompyrimidine were mixed with 10 grams of aniline, heat was evolved and the mass solidified to a hard cake. This was powdered and boiled with benzene, the benzene and aniline were removed by a current of steam and the insoluble material was crystallized from alcohol. It formed a felted mass of colorless needles, difficultly soluble in water, which melted to a clear oil at 191°.

	Calculated for $C_{16}H_{13}N_4Br$.	Found.
N	16.42	16.42

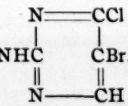
2-Anilino-5-brom-6-oxypyrimidine,



—Five grams of 2-ethylmercapto-5-brom-6-oxypyrimidine were heated with excess of aniline on the steam-bath, until the odor of mercaptan disappeared (24 hours). At first solution took place, then crystalline material separated. The product, after washing with cold alcohol, was free from sulphur and weighed about 6 grams. It was difficultly soluble in water, alcohol, benzene and acetone. From alcohol it separated in the form of colorless, needle-like prisms, melting at 246° with effervescence.

	Calculated for $C_{10}H_8ON_3Br$.	Found.
N	15.78	16.11

2-Anilino-5-brom-6-chlorpyrimidine,

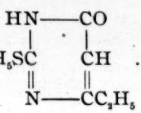


—The above anilinobromoxypyrimidine (4.7 grams) was digested on the steam-bath with phosphorus oxychloride until solution took place and hydrogen chloride ceased being

evolved. It was then poured on to ice and the solid that separated was extracted with ether. It was readily soluble in ether and benzene and crystallized from ligroin in bunches of long, radiating, colorless needles which melted, to a clear oil, at 106°–107°.

	Calculated for $C_{10}H_7N_2ClBr$.	Found.
N	14.76	14.45

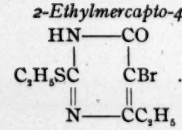
A small quantity of this compound was warmed with excess of aniline on the steam-bath, the mixture liquefied and in a few minutes solidified to a hard cake. On crystallizing from alcohol it gave a felted mass of needles, melting at 191°, and, when mixed with 2,6-dianilino-5-brompyrimidine, described above, the melting-point was not altered.

2-Ethylmercapto-4-ethyl-6-oxypyrimidine,  .

—A solution of 5.7 grams of sodium hydroxide, 12.5 grams of pseudoethylthiourea hydrobromide and 10 grams of ethyl propionyl acetate was made in 25 cc. of water, the constituents being added in the order named. The mixture was allowed to stand for a number of hours, then warmed and, on cooling, it was acidified with acetic acid. An oil separated which finally solidified, and, after washing and drying, weighed about 6 grams. It dissolved, with difficulty, in hot water, giving colorless, truncated pyramids. From alcohol, in which it is very soluble, it crystallized in large, stout, transparent prisms, which melted at 89° without decomposition.

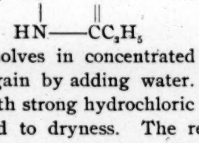
	Calculated for $C_8H_{12}ON_2S$.	Found.
N	15.21	15.13

2-Ethylmercapto-4-ethyl-5-brom-6-oxypyrimidine,

 .—Six grams of the above crude product were dissolved in 8 grams of glacial acetic acid and a slight ex-

cess of bromine added. A mass of six-sided plates separated and more was obtained on diluting with water. The yield of crude material was a little over 6 grams. When crystallized from alcohol it formed large, stout, colorless, transparent prisms and melted, to a clear liquid, at 172°–173°.5.

	Calculated for $C_8H_{11}ON_2SBr$.	Found.
N	10.64	10.64

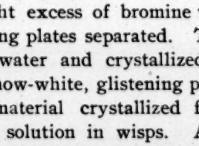


4-Ethyluracil, .—The above mercaptobrom-

pyrimidine dissolves in concentrated hydrochloric acid but is precipitated again by adding water. It was digested on the steam-bath, with strong hydrochloric acid, for some time and then evaporated to dryness. The residue was more readily soluble in water than in alcohol. When crystallized from the latter it formed beautiful, colorless tables or blocks, which melted at 204° to a clear liquid; it effervesced at about 270°.

	Calculated for $C_6H_8O_2N_2$.	Found.
N	20.0	20.2

The same product was obtained by boiling 2-ethylmercapto-4-ethyl-6-oxypyrimidine, melting at 89°, with hydrochloric acid.



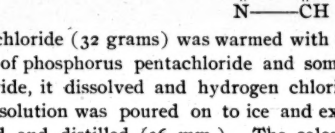
4-Ethyl-5-bromuracil, .—The above 4-ethyl-

uracil was dissolved in glacial acetic acid, in which it is not very soluble and a slight excess of bromine was added. On cooling, very thin, oblong plates separated. These were difficultly soluble in hot water and crystallized, when slowly cooled, in long, thin, snow-white, glistening prisms that were highly etched. The material crystallized from alcohol in plates, or from strong solution in wisps. At 230°–231° it melted to a yellow liquid, which immediately turned brown and then effervesced.

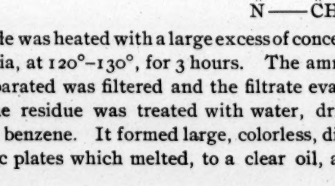
	Calculated for $C_6H_7O_2N_2Br$.	Found.
N	12.78	12.62

2-Methylmercapto-6-oxypyrimidine Hydrochloride.—This was obtained on passing chlorine into a glacial acetic acid solution of the mercaptoxypyrimidine and precipitating with ether. It was crystallized from glacial acetic acid and formed prismatic plates, which were washed with ether. It was very soluble in alcohol and water, the aqueous solution giving a strong odor of mercaptan. When boiled with hydrochloric acid, uracil was obtained. It melted, with vigorous effervescence, at 189°.

	Calculated for $C_5H_6ON_2S.HCl$.	Found.
N	15.7	15.8

2-Methylmercapto-6-chlorpyrimidine,  .—When the above hydrochloride (32 grams) was warmed with the calculated quantity of phosphorus pentachloride and some phosphorus oxychloride, it dissolved and hydrogen chloride was given off. The solution was poured on to ice and extracted with ether, dried and distilled (36 mm.). The colorless oil boiled, for the most part, at 139°–140°; on cooling in a freezing mixture it solidified and melted at about —2°—0°.

	Calculated for $C_5H_5N_2SCl$.	Found.
N	17.4	16.9

2-Methylmercapto-6-aminopyrimidine,  .—The above chloride was heated with a large excess of concentrated alcoholic ammonia, at 120°–130°, for 3 hours. The ammonium chloride that separated was filtered and the filtrate evaporated to dryness. The residue was treated with water, dried and crystallized from benzene. It formed large, colorless, diamond-shaped, prismatic plates which melted, to a clear oil, at 125°–

126°. It was readily soluble in alcohol and acetone, insoluble in ether and ligroin.

	Calculated for $C_6H_7N_3S$.	Found.
N	29.79	29.76

NEW HAVEN, CONN.,
January, 1905.

Contributions from the Sheffield Laboratory of Yale University.

CXXIII. — RESEARCHES ON PYRIMIDINES: THE ACTION OF POTASSIUM THIOCYANATE UPON SOME IMIDE CHLORIDES.

[NINTH PAPER.]

BY HENRY L. WHEELER AND H. STANLEY BRISTOL.

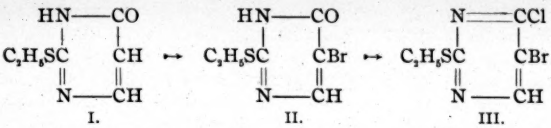
No attention has hitherto been given to the action of potassium thiocyanate upon imide chlorides. It was, therefore, impossible to predict whether normal thiocyanates, RSCN, or isothiocyanates, mustard oils, RNCS, would be formed. Most alkyl halides give normal thiocyanates, while the acyl halides give isothiocyanates. Imide chlorides, $-C\overset{N-}{\underset{Cl}{<}}$, are more closely related to acyl halides, $-C\overset{O}{\underset{Cl}{<}}$, than to the alkyl halides and, in the pyrimidine series, the compounds contain the grouping C=C—CCl,[1] therefore isothiocyanates might be expected to be formed in this reaction. This being the case, facilities for further syntheses would be increased.

We have investigated this reaction in the case of 2-ethylmercapto-6-chlorpyrimidine, 2-ethylmercapto-5-brom-6-chlorpyrimidine and 2-ethylmercapto-5-methyl-6-chlorpyrimidine and we find, in each instance, that the chlorides give isothiocyanates.

The preparation of derivatives has been more thoroughly carried out in the case of 2-ethylmercapto-5-brom-6-chlorpyrimidine, III., than with the other chlorides. This was prepared, as already shown,[2] from 2-ethylmercapto-6-oxypyrim-

[1] Compare Wheeler and Jamieson: J. Am. Chem. Soc., **24**, 746 (1902).
[2] Wheeler and Johnson: THIS JOURNAL, **31**, 603 (1904).

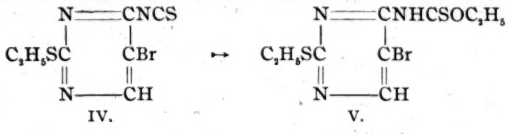
idine (I.) by brominating (II.), then chlorinating in the 6-position.



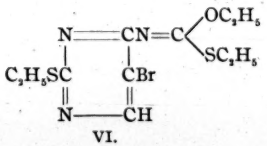
I. II. III.

When 2-ethylmercapto-5-brom-6-chlorpyrimidine was boiled, in toluene solution, with potassium thiocyanate, 2-ethylmercapto-5-brom-6-isothiocyanpyrimidine (IV.) was obtained. This reacted with ammonia, aniline, etc., yielding the corresponding thiourea derivatives.

When, however, the chlorbrommercaptopyrimidine (III.) was boiled with an alcoholic solution of potassium thiocyanate, the thionurethane (V.) was obtained and this compound, with sodium ethylate and ethyl bromide, gave the imidothiocarbonic ester represented by Formula VI.



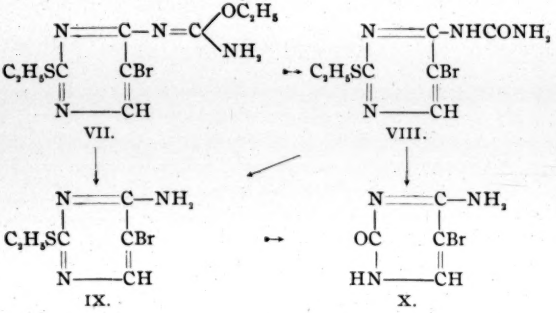
IV. V.



VI.

When this imidothiocarbonic ester derivative was treated with alcoholic ammonia, it evolved mercaptan and the pseudooxygen urea (VII.) was formed smoothly. This gave the normal urea (VIII.) when treated with cold, concentrated hydrochloric acid. Boiling hydrochloric acid then gave 5-bromcytosine (X.). Attempts to condense the pseudooxygen urea, or the normal urea, to a purin derivative were without success. The bromine in these compounds is firmly bound but the urea and pseudourea groups are easily removed.

When the pseudourea (VII.) was heated with pyridine or aqueous ammonia, in a closed tube, 2-ethylmercapto-5-brom-6-aminopyrimidine was formed (IX.). The same result was obtained when the compound was boiled with formic acid. Sodium, in boiling benzene, failed to remove bromine but attacked the pseudourea grouping. 2-Ethylmercapto-5-brom-6-aminopyrimidine was also obtained when the normal urea was heated above its melting-point.

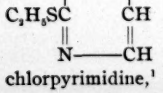


VII. VIII. IX. X.

In the latter part of this article some new uracil and cytosine derivatives are described.

EXPERIMENTAL.

2-Ethylmercapto-6-isothiocyanpyrimidine,



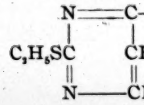
.—Fifteen grams of 2-ethylmercapto-6-chlorpyrimidine,[1] boiling at 135° (24 mm.) and at 128° (16 mm.) were dissolved in 20 cc. of toluene and 25 grams of dry, powdered potassium thiocyanate were added. The mixture was boiled, in a paraffin-bath, for 12 hours. The insoluble salts were then removed by filtering, the toluene was evaporated and the residue distilled under reduced pressure. It boiled, for the most part, at 180° (32 mm.). The distillate solidified

[1] Wheeler and Johnson: THIS JOURNAL, **29**, 497 (1903); **31**, 596 (1904).

and, when crystallized from benzene, it separated in small, light-yellow, prismatic plates and melted, on slowly heating, at 175°.

	Calculated for $C_7H_7N_3S_2$.	Found.
N	21.32	21.53

2-Ethylmercapto-6-thioureapyrimidine,



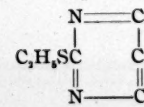
.—On adding concentrated aqueous ammonia to a portion of the above toluene solution containing the isothiocyanate, an energetic reaction took place, with evolution of heat and a solid mass separated. When this material was crystallized from alcohol it formed long, thin, colorless prisms and melted at 214°. It was difficultly soluble in water.

	Calculated for $C_7H_{10}N_4S_2$.	Found.
N	26.17	25.83

2-Ethylmercapto-6-phenylthioureapyrimidine was prepared by adding aniline to a toluene solution of the pure crystallized isothiocyanate. A solid separated, with evolution of heat and, when crystallized from alcohol, it formed a felted mass of long, colorless needles resembling cotton. It was almost insoluble in hot water and fairly soluble in hot alcohol, it melted at 205°.

	Calculated for $C_{13}H_{14}N_4S_2$.	Found.
N	19.31	19.10

2-Ethylmercapto-6-thionethylurethanepyrimidine,



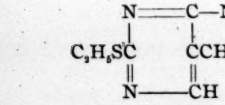
.—When 5 grams of 2-ethylmercapto-6-chlorpyrimidine were added to an alcoholic solution of 2.8 grams of potassium thiocyanate, potassium chloride separated almost at once. The mixture was warmed on the water-bath for 3 hours, the alcohol was then evaporated and the residue treated with water. The crude product weighed 6

grams and, when crystallized from alcohol, it formed long, thin, light-yellow, etched plates, melting at 93°. It is readily soluble in hot alcohol and difficultly soluble in cold.

	Calculated for $C_9H_{12}ON_2S_2$.	Found.
N	17.28	16.89

When this urethane was boiled with concentrated hydrochloric acid, until mercaptan ceased being evolved and then evaporated, a mixture of about equal quantities of uracil and cytosine was obtained.

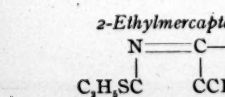
2-Ethylmercapto-5-methyl-6-thioureapyrimidine,



.—Eleven grams of 2-ethylmercapto-5-methyl-6-chlorpyrimidine, 30 grams of dry, finely powdered potassium thiocyanate and 30 cc. of toluene were mixed and boiled for 7 hours, in an oil-bath. The potassium thiocyanate was added, in portions of 10 grams, from time to time. The mixture was filtered and, to a portion of the solution, alcoholic ammonia was added, the thioureapyrimidine then separated in a fairly pure state. It was moderately soluble in hot alcohol and benzene, difficultly soluble in cold and crystallized from benzene in bunches of radiating needles. It was difficultly soluble in hot water and melted, to a clear oil, at 192°.

	Calculated for $C_8H_{12}N_4S_2$.	Found.
N	24.56	24.30

2-Ethylmercapto-5-methyl-6-thionurethanepyrimidine,

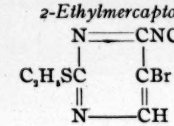


.—Ten grams of the chloride were boiled with 15 grams of dry, finely powdered potassium thiocyanate, in toluene, for 15 hours. Then an excess of alcohol was added and the mixture boiled again for 6 hours. The solution was filtered hot and evaporated to dryness, the residue

treated with water and filtered. It was then taken up in alkali and extracted with ether, the alkaline solution acidified with acetic acid and the pasty, semi-solid precipitate filtered. This was dissolved in ether and dried with potassium carbonate. On evaporation an oil was obtained which solidified completely only after standing 9 months. It was purified by dissolving in benzene and precipitating with ligroin, whereupon groups of pale-yellow prisms separated. It melted at 88°–89°, to a clear oil. It was readily soluble in alcohol, ether and benzene and difficultly soluble in water.

	Calculated for $C_{10}H_{16}ON_2S_2$.	Found.
N	16.34	16.71

2-Ethylmercapto-5-brom-6-isothiocyanpyrimidine,



C_2H_5SC (N=CNCS; CBr; N—CH) . — The 2-ethylmercapto-5-brom-6-chlorpyrimidine, used as the starting-point for the following experiments, was described in a previous paper,[1] but the boiling-point of the material was not given. It was observed to boil at 168° (24-25 mm.) and at 179°–180° (36 mm.). Fifteen gram portions of the chloride were dissolved in 30 cc. of toluene and 30 grams of finely pulverized, dry potassium thiocyanate were added, in portions of 10 grams each, from time to time. The mixture was boiled for 9 hours, in an oil-bath. It was then filtered and the toluene mostly removed by evaporating on the water-bath, under diminished pressure. Petroleum ether was now added, in slight amount, enough to separate an oily substance. The solutions were quickly poured off from this through a filter and, on standing, beautiful, small, pale-yellow prisms formed, which were purified by dissolving in toluene and adding a small amount of petroleum ether. The material was readily soluble in benzene and toluene and difficultly soluble in petroleum ether. It melted at 79°–80°.

	Calculated for $C_7H_9N_3S_2Br$.	Found.
N	15.21	15.45

[1] Wheeler and Johnson: THIS JOURNAL, 31, 603 (1904).

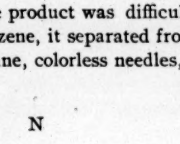
2-Ethylmercapto-5-brom-6-thioureapyrimidine.—A portion of the toluene solution of the isothiocyanate was mixed with alcoholic ammonia in excess. Heat was evolved and the thiourea separated at once. It was found to be difficultly soluble in water and alcohol and more soluble in benzene, from which it crystallized in light-yellow plates, melting at 220°.

	Calculated for $C_7H_9N_4S_2Br$.	Found.
N	19.11	18.96

2-Ethylmercapto-5-brom-6-phenylthioureapyrimidine.—Aniline acted at once, with evolution of heat, on the isothiocyanate. The product was difficultly soluble in water, fairly soluble in benzene, it separated from alcohol in the form of a felted mass of fine, colorless needles, which melted at 166°–167°.

	Calculated for $C_{13}H_{13}N_4S_2Br$.	Found.
N	15.17	15.03

2-Ethylmercapto-5-brom-6-thionethylurethanepyrimidine,



.—The action of 2-ethylmercapto-5-brom-6-chlorpyrimidine on potassium thiocyanate, in alcoholic solution, was not smooth. In addition to the thionurethane, two other compounds were isolated as products of the reaction. These were 2-ethylmercapto-5-brom-6-thiopyrimidine and 2-ethylmercapto-5-brom-6-aminopyrimidine.

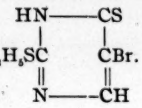
Thirty-three grams of 2-ethylmercapto-5-brom-6-chlorpyrimidine were added to a concentrated solution of 20 grams of potassium thiocyanate in alcohol and the mixture was boiled for several hours. At the end of this operation the odor of ethylthiocyanate was noticed. The solution was filtered and the alcohol evaporated. The residue was treated with cold benzene and filtered, leaving 2-ethylmercapto-5-brom-6-thiopyrimidine and potassium thiocyanate undissolved. The benzene was then evaporated and the residue treated with dilute sodium hydroxide and extracted with ether. The ether solution con-

tained the 2-ethylmercapto-5-brom-6-aminopyrimidine, melting at 124°, which was described in a previous paper.[1]

The urethane was precipitated from the alkaline solution by means of acetic acid; the yield of crude material was 25 grams. It was readily soluble in hot alcohol, difficultly soluble in cold and formed long, pointed, light-yellow, prismatic plates, melting at 82°.

In two other experiments 18.5 grams of the chloride gave 13 grams of the urethane and 15 grams of the chloride gave 11.5 grams of the urethane.

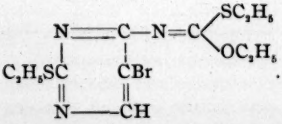
	Calculated for $C_9H_{12}ON_2S_2Br$.	Found.
N	13.04	12.97

2-Ethylmercapto-5-brom-6-thiopyrimidine, 

—The residue, left undissolved by cold benzene, in the above preparation was washed with water, dried and crystallized from benzene. It formed pointed, etched, pale-yellow plates and melted, with slight effervescence, at 198°.

	Calculated for $C_6H_7N_2S_2Br$.	Found.
N	11.15	11.04

In the course of some other work Mr. S. H. Clapp prepared this compound, by the action of potassium hydrosulphide on 2-ethylmercapto-5-brom-6-chlorpyrimidine. When this preparation was mixed with the above the melting-point was not altered.

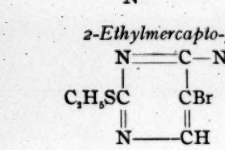
2-Ethylmercapto-5-brom-6-iminothioethylcarbonatepyrimidine,  .—Six grams of the above thionethylurethane were dissolved in a solution of 0.42 gram of sodium, in a small quantity of absolute alcohol and then 4 grams

[1] THIS JOURNAL, 31, 604 (1904).

of ethyl bromide were added. On standing a short time sodium bromide separated. The mixture was warmed on the steam-bath, then filtered and evaporated to dryness. The product was treated with ether, filtered from sodium bromide and evaporated. The residue, on cooling, solidified. The yield was found to be 6 grams. The product was purified by crystallizing from alcohol containing a little water. It then formed long, colorless prisms, melting at 43°, which were readily soluble in alcohol, ether and warm ligroin but insoluble in water.

	Calculated for $C_{11}H_{16}ON_3S_2Br$.	Found.
N	12.00	11.87

2-Ethylmercapto-5-brom-6-pseudoethylureapyrimidine,



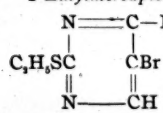
.—This was formed, quantitatively, by passing ammonia gas into an alcoholic solution of the above aminothioethylcarbonate. It crystallized from alcohol in beautiful, long needles, which developed into thin plates, melting at 110° and decomposing about 230°, forming a tarry mass on cooling. It was difficultly soluble in hot water and insoluble in cold, dissolved readily in cold benzene and warm alcohol. It was insoluble in alkali and soluble in hydrochloric acid.

	Calculated for $C_9H_{13}ON_4SBr$.	Found.
N	18.3	18.1

This material can be boiled with pyridine (3 hours) or dimethyl aniline (10 minutes) and recovered unaltered. No bromine is removed under these conditions. When heated with pyridine at 180°–190°, in a closed tube, for 5 hours, the only crystalline substance obtained was 2-ethylmercapto-5-brom-6-aminopyrimidine. This substance was also obtained when the material was boiled with formic acid. A nitrogen determination gave 17.7 per cent, while the calculated is 17.9 per cent. The action of concentrated aqueous ammonia, in a closed tube, first at 90° for 3 hours, then at 145°–150° for 4½

hours, gave the same result. The hydrochloride of this base was obtained when the pseudourea was warmed, for a short time, with concentrated hydrochloric acid. About 0.5 gram of the pseudourea was dissolved in benzene and treated with an excess of sodium, the mixture was boiled for 12 hours. The metal became coated with a thick deposit and, on filtering and evaporating the benzene, there was no residue. The sodium and coating was treated with alcohol and a portion was acidified with nitric acid, whereupon silver nitrate gave no insoluble precipitate, and, therefore, no bromine had been removed. On acidifying the alkaline solution with acetic acid and evaporating, a substance was obtained melting at 287°, with effervescence. On dissolving in acetone and precipitating with ether it effervesced at 310°. It was concluded that this material was *2-ethylmercapto-5-brom-6-cyanamidepyrimidine*, not only from its mode of formation, but also because, on warming with dilute hydrochloric acid, it gave the following urea derivative:

2-Ethylmercapto-5-brom-6-ureapyrimidine,



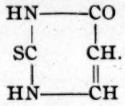
.—This was also produced by allowing the above pseudourea to stand dissolved in cold concentrated hydrochloric acid. After several days the urea separated on adding water. It crystallized from alcohol in short, pointed plates which, on long standing, developed into stout prisms and melted, when slowly heated, at 167°.

	Calculated for $C_7H_9ON_4SBr$.	Found.
N	20.2	19.8

If this material is heated to its melting-point, cooled and reheated, it melts unaltered. If the heating is continued a little above its melting-point it slowly becomes semi-solid. One gram of the urea derivative was heated, in a test-tube, at 170°–180°, a portion sublimed, crystallizing in prismatic plates in the cool part of the tube. This melted at 124° and, when

mixed with some 2-ethylmercapto-5-brom-6-aminopyrimidine, the melting-point was not altered.

When this urea was boiled with concentrated hydrochloric acid 5-bromcytosine[1] was obtained.



2-Thiouracil, —This compound was first obtained, along with 2-ethylmercapto-6-oxypyrimidine, when pseudoethylthiourea, containing some thiourea, was condensed with ethyl sodium formylacetate. A concentrated aqueous solution of 150 grams of ethyl sodium formylacetate was mixed with a saturated solution of 50 grams of thiourea. The mixture was then warmed on the steam-bath for a short time, cooled and the deep red solution was precipitated with a slight excess of acetic acid. A yellowish-white, granular precipitate separated, weighing 50 grams. This crystallized from alcohol or water in colorless prisms. It is difficultly soluble in these solvents. When heated, it effervesces above 300°.

	Calculated for $C_4H_4ON_2S$.	Found.
N	21.8	21.9

2-Ethylmercapto-6-anilinopyrimidine hydrochloride was prepared by mixing 2-ethylmercapto-6-chlorpyrimidine (10 grams) with aniline (5.3 grams), after standing over night, then warming on the water-bath for several hours, the mixture solidified. It was readily soluble in hot alcohol and hot water and crystallized in the form of colorless needles or prisms. It melted at 198° with effervescence.

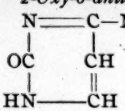
	Calculated for $C_{12}H_{14}N_3SCl$.	Found.
N	15.7	15.9

When this hydrochloride was dissolved in hot water and precipitated with ammonia, an oil separated. This finally solidified and was purified by repeatedly dissolving in alcohol and precipitating with water. The base is very soluble in alcohol. It formed fine, colorless needles and melted at 68°.

[1] THIS JOURNAL, 31, 604 (1904).

	Calculated for $C_{12}H_{12}N_2S$.	Found.
N	18.1	18.2

2-Oxy-6-anilinopyrimidine (*Phenylcytosine*),



.—The hydrochloride of this base was obtained by boiling the above hydrochloride with strong hydrochloric acid. It formed colorless needles or prisms, from water or alcohol, which effervesced at 228°. This material was dissolved in hot water and ammonia was added, whereupon bunches of colorless needles separated. Unlike cytosine, this substance crystallizes without water of crystallization. It is fairly soluble in hot alcohol and, from this solvent, it separates in beautiful, colorless, six-sided plates. When slowly heated it melted at about 269°.

	Calculated for $C_{10}H_{10}ON_2$.	Found.
N	22.3	22.6

The *chlorplatinate* formed groups of radiating yellow prisms. A platinum and water determination agreed with the calculated for a normal salt with 1 molecule of water of crystallization. The formation of platinum chloride double salts with water of crystallization seems to be of frequent occurrence in the pyrimidine series.

	Calculated for $(C_{10}H_{10}ON_2)_2H_2PtCl_6.H_2O$.	Found.
Pt	24.24	24.31
H_2O	0.22	0.26

2,6-Dianilinopyrimidine, C_6H_5NHC (with N, C, CH, N, CH and NHC_6H_5),

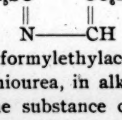
.—The hydrochloride of this base was obtained when 2-ethylmercapto-6-chlorpyrimidine was heated to 150°, with 2 molecular proportions of aniline. The compound formed arborescent needles from alcohol and melted at 197°.

	Calculated for $C_{16}H_{14}N_4Cl$.	Found.
N	18.76	18.59

The free base, obtained by boiling the hydrochloride with aqueous ammonia, crystallized from alcohol in six-sided plates. It is insoluble in water and fairly soluble in hot benzene and ether. It melted at 136°–137°.

	Calculated for $C_{16}H_{14}N_4$.	Found.
N	21.38	21.42

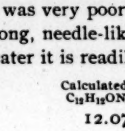
2-Ethylmercapto-5-phenyluracil,



was obtained by condensing sodium phenylformylethylacetate[1] with the ethyl iodide addition-product of thiourea, in alkaline solution. The yield was very poor. The substance crystallized from alcohol in long, needle-like prisms, melting at 158°. In alcohol and hot water it is readily soluble.

	Calculated for $C_{12}H_{12}ON_2S$.	Found.
N	12.07	12.12

5-Phenyluracil,



—When the above compound was digested on the steam-bath, with concentrated hydrochloric acid, for a number of hours, mercaptan was evolved and difficultly soluble material was obtained. The product was sparingly soluble in hot alcohol and water, but it separated from these solutions, on cooling, in a flocculent mass of what appeared to be microscopic plates. It did not melt at 350°. It is soluble in alkali and can be precipitated unaltered by hydrochloric acid.

	Calculated for $C_{10}H_8O_2N_2$.	Found.
N	14.89	14.87

The isomeric 4-phenyluracil has been described by Fischer and Roeder[2] and it was also obtained by one of us.[3]

NEW HAVEN, CONN.,
January, 1905.

[1] Wislicenus: Ber. d. chem. Ges., **20**, 2933 (1887).
[2] *Ibid.*, **34**, 3751 (1901).
[3] Wheeler and Merriam: THIS JOURNAL, **29**, 490 (1903).

Contributions from the New York Agricultural Experiment Station.

X.—CASEIN AND PARACASEIN IN SOME OF THEIR RELATIONS TO BASES AND ACIDS.

By Lucius L. Van Slyke and Edwin B. Hart.

For several years we have been studying the chemical changes that occur in the process of cheese-making and cheese-ripening. Early in our work we extracted fresh and partially ripened cheese with dilute solution of sodium chloride, following a suggestion of Chittenden,[1] who had shown that, in a peptic digestion of casein, heterocaseose was formed only in small amounts.

As we obtained, by our extraction with dilute salt solution, amounts of proteid representing often as much as 75–80 per cent of the total nitrogen present in the cheese, it became apparent that we were dealing with some compound other than heterocaseose. The first suggestion, throwing any light on the method of formation of this salt-soluble substance, came in the following manner: In studying the action of galactase in cheese-ripening, we made cheddar cheese from milk containing chloroform, added for the purpose of inhibiting bacterial action. Under these conditions no lactic acid was present. In order to approximate more closely to the conditions present in normal cheddar cheese we added, in a parallel experiment, a small quantity of lactic acid, the other conditions being kept uniform. We noticed at once that, when no acid was present in the cheese, we found little or no salt-soluble substance, while in the cheese containing a little added lactic acid there were present marked quantities of the salt-soluble product. This fact suggested that lactic acid was a necessary agent in forming the salt-soluble proteid found by us in cheese. Hence we were led to believe that there is a chemical combination between the paracasein of the fresh cheese-curd and the lactic acid formed from milk-sugar during the cheese-making process. We were also able to form salt-soluble proteids by treating paracasein with such acids as acetic, hydrochloric and sulphuric. In each case definite amounts of acid disappeared

[1] "Studies in Physiol. Chem.," Yale Univ., **2**, 156 (1885–6).

and the proteid treated underwent marked and definite changes in properties. By treating the salt-soluble compound with about the same amount of acid as was required to form it from paracasein, we obtained a body insoluble in salt solution and differing also in other properties. We were thus led to conclude that two series of salts were formed by treating paracasein with acids, one containing twice as much acid in combination as the other. We therefore called the salt-soluble compound, found in normal cheddar cheese, paracasein monolactate and the one insoluble in salt solution, paracasein dilactate. We also found the same behavior in the case of milk-casein when treated by acids. The two series of compounds varied in respect to many of their properties.[1] Our conclusions were based upon the fact that, when casein or paracasein is treated with an acid in definite quantities, we obtain compounds of definitely characteristic properties.

We desired, however, if possible, to carry our work farther and prove, beyond question, that there is an actual chemical combination between acid and proteid. We wished to prepare the free proteid and then treat this with acid and study the results.

Hammarsten[2] made a special study of the interaction of casein and acids and concluded that there was no ground for believing that a chemical combination takes place between casein and the acid used to precipitate it. He based his statement on the fact that, by triturating for several days, in a mortar, with different portions of water, a precipitate formed by casein with an acid, he was able to remove the acid so completely that the remaining precipitate gave no test for it. The substance which he used in his work was what we have called a di-acid compound of casein. In repeating Hammarsten's work we were able to obtain a substance free from the acids used as precipitants and also practically free from ash. We found this substance, when freshly prepared, readily soluble in warm dilute salt solution and in hot dilute alcohol; it also showed the ductile and plastic properties of the substance we called a mono-salt of casein. The facts, as they presented

[1] This Journal, **28**, 411–438 (1902).

[2] Maly: Jahres. d. Thierchemie, **7**, 160 (1877).

themselves to us at this point, were as follows: A precipitate, formed by treating milk-casein with an acid, being what we called a di-salt of casein, was, by continuous washing and trituration, converted into a substance free from the acid used as precipitant and nearly free from ash; the substance precipitated by acid, insoluble in warm dilute salt solution and in hot 50 per cent alcohol, was, by continuous trituration under water and washing, converted into a substance easily soluble in warm dilute salt solution and in hot 50 per cent alcohol. There was also a simultaneous change in other properties. Thus, what we called a di-salt of casein was changed into what we had termed a mono-salt of casein, but the latter was free from acid and ash.

In our early study we recognized, in a limited way, the relation of inorganic salts in milk to the neutralization of acid. We noticed that when milk, coagulated by rennet enzyme, is treated with varying amounts of acid, the quantity of salt-soluble substance did not increase until a certain amount of acid had been added, after which it increased quite out of proportion to the larger amount of acid used. It was suggested that the formation of the salt-soluble compound did not begin until certain inorganic salts of the milk had been neutralized.[1]

After developing the later facts described above, we decided to study more fully the following questions:

1. What is the relation between milk-casein and the salt-soluble compounds which we have called casein mono-salts?
2. What is the relation between the two series of compounds previously called casein mono-salts and casein di-salts?
3. What is the relation of casein and its derivatives to paracasein and its derivatives?

It may be well to state, once for all, that our work deals exclusively with the casein of cows' milk and the products derived from it.

The Relation of Milk-Casein to the Casein Compounds Soluble in Dilute Salt Solutions.

A study of the relation of milk-casein to the salt-soluble substance, which we have previously called a casein mono-salt,

[1] THIS JOURNAL, 28, 428 (1902).

naturally resolves itself into a study of the two following questions: (1) What is milk-casein, especially in relation to inorganic compounds? (2) What is the salt-soluble compound formed from milk-casein?

The Relation of Inorganic Compounds to Milk-Casein.—Does casein, as it is found in cows' milk, exist as a free proteid, or is it in some form of combination with an inorganic compound?

Hammarsten, who must be regarded as the most successful pioneer worker in this field, has not clearly and specifically stated what he considers milk-casein to be. He does not appear to make any chemical distinction between ash-free casein, as prepared from cows' milk by his well-known method and casein as it is found in milk. He appears to favor the idea that there is, in milk-casein, some kind of physical relation between the proteid and calcium phosphate, casein acting as a solvent for calcium phosphate.[1] Eugling[2] held that casein enters into combination with tricalcium phosphate and the same view was held by Schaeffer.[3] Söldner[4] showed that casein, prepared from cows' milk, can form two calcium salts, (1) a basic calcium casein, containing about 2.36 per cent of calcium oxide, neutral to phenolphthalein, but alkaline to litmus; (2) a neutral calcium casein, containing about 1.55 per cent of calcium oxide, slightly acid to phenolphthalein and neutral or slightly alkaline to litmus. Söldner believed that this neutral calcium casein is probably the form existing in milk, owing to its reaction and to its coagulability by rennet in the presence of soluble calcium salts. He also held that calcium phosphate, usually found in casein precipitates, exists in a condition of suspended particles, finely divided and not in chemical combination with casein. Courant[5] confirmed the work of Söldner, calling the basic calcium compound tri-calcium casein and the neutral compound di-calcium casein. He also reported, on what seems to us as rather intangible evidence, a mono-calcium casein, the existence of which has not been confirmed and appears doubtful. He regards milk-casein as made up partly of

[1] Maly: Jahres. d. Thierchemie, **4**, 146 (1874).
[2] Landw. Versuchs-Stat., **31**, 392 (1885).
[3] Landw. Jahrb. d. Schweiz, **1**, 33 (1887).
[4] Landw. Versuch-Stat., **35**, 351 (1888).
[5] Pflügers Arch., **50**, 109 (1891).

di- and partly of mono-calcium casein. Courant's work was entirely volumetric and no attempt was made to isolate the individual compounds. Lehman and Hempel[1] regard milk-casein as a double compound, formed by the combination of calcium casein and calcium phosphate.

Our work confirms Söldner's, which was carried on mostly by volumetric methods, whereas we have succeeded in isolating the two calcium casein compounds described by him.

Preparation of Casein.—We prepared caesin by Hammarsten's method, repeating the precipitation and solution 4 times. The precipitate was finally dissolved in 20 liters of very dilute ammonia water and precipitated by dilute acetic acid. The precipitate was ground in a mortar several times, with water, then with alcohol, after which it was treated with ether and dried at 100°. Thus prepared, the casein contained only 0.042 per cent of ash. The preparation was still slightly soluble in warm salt solution and in hot 50 per cent alcohol.

Behavior of Casein Preparation toward Calcium Carbonate.—We rubbed in a mortar 0.5 gram of the casein preparation described above with finely divided calcium carbonate, suspended in water. The mixture was filtered several times through paper, giving a slightly opalescent solution. This solution was precipitated by 95 per cent alcohol, the precipitate was washed several times with alcohol, then treated with ether and dried at 100°. The product contained 2.46 per cent of calcium oxide, agreeing very closely with Söldner's work, in which he showed, by an indirect method, that a casein preparation, treated with calcium carbonate, combined with 2.39 per cent of calcium oxide. A part of this calcium casein preparation was dissolved in dilute ammonia, reprecipitated by dilute acetic acid and the precipitate, after thorough washing and filtering, was again obtained practically ash-free.

Solubility of Casein Preparation in Lime-Water.—We dissolved 3 grams of our original casein preparation in lime-water and obtained an opalescent solution.

Preparation of Basic Calcium Casein.—The lime-water solution of casein was made neutral to phenolphthalein by N/10

[1] Pflügers Arch., 56, 558 (1894).

hydrochloric acid and became somewhat more milky. This solution was treated with 95 per cent alcohol and the resulting precipitate, which readily separated, was washed several times with alcohol, finally with ether and dried at 100°. This precipitate contained 0.75–1.2 per cent of calcium chloride, either as the result of occlusion or, possibly, of a loose combination with the proteid. Chlorine and calcium determinations were made with the following results: 1.24 grams of material yielded 0.0331 gram of CaO. There was present 0.0063 gram of Cl, equivalent to 0.0097 gram of $CaCl_2$, or 0.0047 gram of CaO. This would leave 0.0284 gram of CaO combined with casein, or 2.29 per cent. The compound was, therefore, the basic calcium casein of Söldner. It was insoluble in 5 per cent salt solution and in hot 50 per cent alcohol.

Preparation of Neutral Calcium Casein.—When a solution of casein in lime-water is made neutral to phenolphthalein, the basic calcium casein is formed containing about 2.40 (2.29–2.46) per cent of calcium oxide. When the addition of acid is carried farther, a point is reached where the solution becomes neutral to litmus and much more milky in appearance than the solution neutral to phenolphthalein. A solution of casein in lime-water was made neutral to litmus by the addition of N/10 hydrochloric acid; to this solution alcohol was added, throwing down a white precipitate. The precipitate was washed with alcohol and ether and dried at 100°. It contained 1.13 per cent of calcium chloride. Analysis: 1.27 grams gave 0.0257 gram of CaO and 0.0093 gram of Cl, equivalent to 0.0072 gram of CaO, leaving 0.0185 gram of CaO or 1.46 per cent as combined with the proteid. Söldner recognized this combination and attributed to it a content of 1.55 per cent of calcium oxide. Another preparation contained 0.86 per cent of calcium chloride and 1.48 per cent of calcium oxide combined with casein.

Behavior of Calcium Casein Solution toward Rennet Enzyme and Calcium Chloride.—A solution of basic calcium casein, the compound neutral to phenolphthalein, is not coagulated by rennet enzyme either in the presence or absence of soluble calcium salts, such as calcium chloride. A solution of neutral calcium

casein, the compound neutral to litmus, is coagulated by rennet in the presence of soluble calcium salts. When soluble calcium salts are removed by ammonium oxalate, no coagulation occurs. These statements are in agreement with the observations of others.

Casein Present in Milk as Neutral Calcium Casein.—Our results appear to confirm, in every respect, the work of Söldner in regard to the relation of inorganic compounds to milk-casein. We have had the advantage of isolating the two compounds, which with him were more largely matters of inference. Of these two calcium casein derivatives, the one containing about 1.55 per cent of calcium oxide, neutral to litmus and coagulable by rennet enzyme in the presence of soluble calcium salts, appears to meet all the requirements of milk-casein.

The Behavior of Calcium Casein Compounds toward Soluble Calcium Salts.—Basic calcium casein, prepared by dissolving free casein in lime-water and making neutral to phenolphthalein by acid, is coagulated by soluble calcium salts on warming at 35°–40°. The coagulum is insoluble in warm, 5 per cent salt solution. If any soluble calcium salts present are removed by treatment with ammonium oxalate, the basic calcium casein is not coagulated on warming.

Neutral calcium casein, prepared by dissolving free casein in lime-water and making neutral to litmus, is coagulated on warming by soluble calcium salts. The coagulum is insoluble in warm dilute salt solution. The neutral calcium casein existing in milk is coagulated, on warming, by a few drops of calcium chloride solution. If we add to milk an amount of acid insufficient to cause coagulation, or to remove from its combination the calcium of the calcium casein, we can produce some coagulation on warming. This is probably due to the conversion of insoluble into soluble calcium salts and the same result follows as when we add a soluble calcium salt directly to the milk.

The coagulation of calcium casein compounds, by a soluble calcium salt, may be due to purely physical change in the calcium casein compounds, or there may be a loose chemical com-

bination between the soluble calcium salt and the calcium casein derivatives, the resulting compound being insoluble in the neutral or slightly acid medium.

The Relation of the Salt-Soluble Compound of Casein to Milk-Casein.—The preparations of calcium casein, while slightly soluble in hot 50 per cent alcohol, were insoluble in warm 5 per cent salt solution. None of them showed, in any marked degree, the properties of the compounds that we have called casein mono-salts. We have already called attention to the fact that, in making an ash-free preparation of casein from cows' milk, we found it to be readily soluble in both warm dilute salt solution and in hot 50 per cent alcohol. This suggested that there might be a close relation between these compounds and we therefore made several preparations, the details of which we now give.

Preparation 1, by the Use of Hydrochloric Acid.—Two liters of skimmed milk were diluted with 5 volumes of distilled water and the mixture was warmed to 45°. Dilute hydrochloric acid was added, accompanied by vigorous stirring to keep the precipitate in a finely divided condition. After the precipitate had settled, the supernatant liquid was poured off, the precipitate was triturated in a mortar with water and finally allowed to drain on filter-paper. It was then removed from the filter-paper, mixed with water at 45°–50°, triturated and filtered again. This was repeated 4 or 5 times a day and continued until the filtrate gave no test for chlorides, which required 4 days. When the precipitate had finally been drained it was washed with 95 per cent alcohol. Some of the proteid went into solution, owing to dilution of the alcohol by the water still present in the precipitate and this partial solution stopped only after a large volume of alcohol had been used. The precipitate was finally washed with ether and dried at 100°. The product was a very friable, white powder. It was still somewhat soluble in hot 50 per cent alcohol and warm 5 per cent salt solution. These solubilities are much more marked before treatment with strong alcohol and ether. The product was practically ash-free, 5.28 grams yielding 0.02 per cent of ash. The preparation was quite free from chlorides, this being

shown in the following manner: We dissolved 0.5 gram in hot, concentrated nitric acid, to which was added a crystal of silver nitrate. The whole was boiled until complete solution of the proteid had occurred. No precipitate of silver chloride was formed.

Preparation 2, by Hammarsten's Method.—We used 2 liters of skim-milk and followed in exact detail the method described by Hammarsten.[1] Before treatment with alcohol this preparation was readily soluble in warm dilute salt solution and in hot 50 per cent alcohol. It contained 1.06 per cent of ash. It was shown to be free from acetate in the following manner: We dissolved 0.5 gram in dilute sodium hydroxide, then precipitated the proteid by hydrochloric acid and filtered. The filtrate was made alkaline and evaporated to dryness. The residue gave no evidence of ethyl acetate when heated with alcohol and sulphuric acid.

Preparation 3, by the Use of Hydrochloric Acid.—We used 2 liters of skim-milk, diluting it with 15 liters of distilled water and precipitating the casein by hydrochloric acid. The separated proteid was allowed to drain on filter-paper and washed with water at 45°. It was then mixed with water and triturated and washed several times. The precipitate soon showed marked solubility in warm dilute salt solution and hot 50 per cent alcohol. The washing was continued until all chlorides were removed. The precipitate was finally washed with alcohol and ether and dried at 100°. It was free from chlorides. Its solubility in alcohol or salt solution was now slight. The ash content was 0.81 per cent. We have noticed that the degree of fineness of the proteid, after precipitation, largely influences the ash content.

Preparation 4, by the Use of Sulphuric Acid.—The same method was followed as that described in preparation 3, except that we used sulphuric acid as the precipitant in place of hydrochloric acid. Triturating this preparation, under different portions of water, 4 or 5 times a day, we had to continue the operation 19 days before the filtrate failed to show the presence of sulphuric acid. A little chloroform was added from time to

[1] Maly: Jahres. d. Thierchemie, 7, 159 (1877).

time, to prevent bacterial action. The proteid was found to be entirely free from sulphuric acid. The extreme difficulty in removing the sulphuric acid was due to the formation of calcium sulphate in the first precipitation and to its slow solubility in water. This preparation had an ash content of 0.56 per cent. During the process of removing the sulphuric acid the precipitate was readily soluble in hot 50 per cent alcohol and in warm 5 per cent solution of sodium chloride. After treatment with alcohol these solubilities were almost entirely lost.

Preparation 5, made from Lime-Water Solution of Casein.—If we dissolve an ash-free preparation of casein in lime-water and add N/10 hydrochloric acid until blue litmus is just turned red, no visible separation of proteid occurs. If we continue to add acid the proteid separates and complete precipitation occurs when all the calcium in the original lime-water solution has been neutralized. This can be shown quantitatively in the following manner: Of the lime-water employed, 50 cc. required for neutralization 19.2 cc. of N/10 hydrochloric acid, using phenolphthalein. We dissolved 0.5 gram of ash-free casein in 50 cc. of this lime-water. On titration, the neutral point, with phenolphthalein, was reached when we had used 15.2 cc. of N/10 hydrochloric acid. This means that calcium oxide, equivalent to 4 cc. of N/10 hydrochloric acid, was no longer present as calcium hydroxide, that is, 0.0112 gram of calcium oxide. This amount had combined with the 0.5 gram of proteid, forming 2.24 per cent of the calcium proteid compound and this proportion indicates the basic calcium casein.

When we had added 16.4 cc. of N/10 hydrochloric acid the solution was just blue to litmus. This indicates that calcium oxide, equivalent to 2.8 cc. of N/10 hydrochloric acid, or 0.00784 gram of calcium oxide, was no longer present as calcium hydroxide but had combined with the 0.5 gram of proteid, forming 1.57 per cent of the calcium proteid combination; this proportion indicates the neutral calcium casein.

When we had added 16.6 cc. of N/10 hydrochloric acid to the lime-water solution of casein, litmus was turned red, but no precipitation occurred. After 18.5 cc. of N/10 hydrochloric

acid had been added a precipitate began to form and was complete when we had added 19.2 cc. N/10 hydrochloric acid, or just enough to combine with all the calcium originally present in the solution used. This work was repeated with closely agreeing results. *This precipitate is, therefore, base-free casein.* A preparation, made in this manner and thoroughly washed, showed that all the calcium was present as calcium chloride, either occluded in the precipitate or, possibly, in a loose form of combination with the proteid; in any case there was no calcium oxide in combination with the casein. The precipitate contained, in 1 gram, 0.00343 gram of calcium and 0.0064 gram of chlorine, which amounts agree well for the presence of calcium chloride. This body, prepared by neutralizing a solution of calcium casein, is insoluble in water but readily soluble in 5 per cent solution of sodium chloride and in hot 50 per cent alcohol.

In another experiment, 25 cc. of lime-water required 10.6 cc. of N/10 hydrochloric acid to neutralize it. We dissolved, in this amount of lime-water, 0.5 gram of ash-free casein, prepared according to Hammarsten. To precipitate this quantity of casein completely required 10.6 cc. of N/10 hydrochloric acid. The precipitated substance was soluble in warm dilute salt solution and in hot dilute alcohol. To the compound we added 0.5 cc. of N/10 hydrochloric acid and obtained a substance insoluble in warm dilute salt solution and only slightly soluble in hot dilute alcohol.

When the product, made by neutralizing the lime-water solutions of ash-free casein with hydrochloric acid, was dissolved in hot 50 per cent alcohol, the dissolved proteid separated from the solution on cooling, forming, on the bottom of the beaker, a gummy, sticky mass, which could easily be gathered on the end of a glass rod. When the substance, freshly precipitated from its solution in lime-water, is warmed on the water-bath it can also be easily gathered on a glass rod in an adherent, gummy mass. When warm, it is plastic and can be drawn out in long, fine, silky threads.

Identity of Base-Free Casein and the Salt-Soluble Body.—In the presentation of the preceding facts we have seen that, when an

ash-free, *i. e.*, base-free proteid, or one practically so, is prepared by precipitating milk-casein (calcium casein) with an acid, the acid precipitant being completely removed from the proteid by long-continued trituration in water; or when a base-free proteid is prepared by treating a lime-water solution of a base-free casein with an acid just to the point of acidity with litmus, we obtain a body which is soluble in warm 5 per cent salt solution and in hot 50 per cent alcohol. This substance, when freshly prepared and warmed, is very plastic and is capable of being drawn into very long, fine, silky threads. It behaves in all respects like the compound which we were led to regard, at first, as formed by combination of casein and an acid and which we regarded as a casein mono-salt of the acid precipitant. As a result of this work, we now believe that the compound formed by treating milk with an amount of acid just sufficient to combine with the calcium of the calcium casein, in addition to certain inorganic salts of the milk, is not a casein mono-salt of the acid precipitant but *is base-free casein or calcium casein (milk-casein) from which the calcium has been removed by combination with acid.*

The Relation between the Two Series of Compounds Previously Called Casein Mono-salts and Casein Di-salts.

We have already seen that, when calcium casein (milk-casein) is treated with dilute acid, the base is removed from its combination with the proteid and the base-free proteid is formed, a compound corresponding in its properties to those of the compounds we formerly called casein mono-salts of acids. When, to this base-free proteid, we add a dilute acid, another body appears to be formed, which is insoluble in warm 5 per cent salt solution and in hot 50 per cent alcohol and which differs also in a marked lack of the plastic properties exhibited by the base-free proteid when freshly prepared and warmed. It is the familiar substance ultimately formed when milk is coagulated by ordinary souring, or by direct addition of acids in sufficient quantity. The formation of this substance has usually been explained in two different ways:

(I.) It has been quite generally held that the acid unites with the inorganic portion (calcium) of the milk-casein, thus

destroying the combination the free proteid then appearing as a solid.

(II.) It has been supposed by some that the acid actually combines with the proteid, forming a casein salt of the acid used. According to the first explanation, only one substance is formed when milk-casein is precipitated with an acid and that is the base-free casein. According to the second explanation, two compounds are formed, one after the other, by treating milk-casein with an acid, *viz.*, (1) the base-free casein; (2) a compound formed by the combination of the base-free casein with the acid.

The existence must be acknowledged of one body with two different sets of properties, or of two different bodies, differing in their properties, when we treat milk-casein with an acid in proper proportions, because, by treating calcium casein with a certain amount of acid, we obtain the base-free proteid and then, by treating this with an additional amount of acid, a body differing in properties from the base-free proteid is formed; the difference must either be due to chemical combination of the proteid and acid or else be the result of a purely physical change caused by the acid. Contrary to the view generally held, we have believed that there was actual combination betweed the proteid and acid, forming a casein salt of the acid and this we formerly called a casein di-salt of the acid used. Since we have shown that our supposed casein mono-salts of acids are simply the base-free casein, it would appear that there is only one series of casein compounds formed by combination with acids, existing as a precipitate.

Hammarsten held that there could be no combination between the casein and acid, because, by trituration with water in a mortar, he was able to remove the acid completely from the proteid. We have abundantly confirmed his statement that the acid can be removed from the proteid by long-continued trituration, but, in view of recent developments in chemistry, this argument has no weight, because we can do the same thing in the case of some well-known and well-established inorganic salts. For example, the sulphuric acid radical of mercuric sulphate can be readily and completely removed by triturating with water.

Experiments Showing Combination of Casein and Acids.—In order to show that there is actual combination between base-free casein and acids, we used the following method: We suspended, in 50 cc. of distilled water, 0.5 gram of finely-ground base-free casein and allowed the mixture to stand 1 hour, with occasional shaking. At the end of this time we added hydrochloric acid, of known strength, in definite quantities, after which the volume was made to 100 cc. by water and filtered. The residue was not washed. Aliquot parts of the filtrate were titrated with N/100 sodium hydroxide, using phenolphthalein as indicator. The difference between the amount of acid added and that found in the filtrate represents the amount of acid combining with the casein, plus the amount adhering mechanically to the casein. It was necessary to use dilute solutions of acid in order to avoid dissolving the proteid by an excess of acid. The materials used in this work were the 4 preparations of base-free casein which have already been described on p. 471.

Amount of Acid Used Kept Constant in Relation to Proteid.—In the experiments, the results of which are given below in Table I., we used different solutions of hydrochloric acid of varying dilution, but varied the quantity so that the same absolute amount of acid was used in each case, which was equal to 20 cc. of N/100 hydrochloric acid for 1 gram of base-free casein. The work was carried on at 17°–18°.

Amount of Acid Used Varying in Relation to Proteid.—In another set of experiments we used varying quantities of acid for 1 gram of proteid. Otherwise the operation was carried out like those preceding. The results are given in Table II.

Amount of Acid and Amount of Dilution Varied in Relation to Proteid.—The amount of water, in which the solid, base-free casein was suspended, was relatively considerable in the preceding experiments. We wished to see what difference, if any, there might be when we treated the casein directly with acid of varying strength and without further dilution. We suspended 1 gram of the base-free casein in each of the following amounts of hydrochloric acid: 50 cc. N/200, 40 cc. N/100, 30 cc. N/80, and 20 cc. N/60. The mixtures were allowed to

Table I.—Amount of Hydrochloric Acid Neutralized by One Gram of Base-Free Casein with Constant Amount of Acid.

No. of cc. of acid used.	Strength of acid used.	Preparation 1.		Preparation 2.		Preparation 3.		Preparation 4.	
		N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
40	N/200	13.6	6.4	13.6	6.4	13.8	6.2	14.0	6.0
20	N/100	13.4	6.6	14.2	5.8	14.0	6.0	14.2	5.8
16	N/80	13.6	6.4	13.6	6.4	13.6	6.4	14.4	5.6
12	N/60	14.0	6.0	13.6	6.4	14.0	6.0	14.0	6.0
8	N/40	14.0	6.0	14.0	6.0	14.0	6.0	14.0	6.0
4	N/20	13.6	6.4	14.0	6.0	14.0	6.0	14.2	5.8
	Average,	13.7	6.3	13.8	6.2	13.9	6.1	14.1	5.9

Table II.—Amount of Hydrochloric Acid Neutralized by One Gram of Base-Free Casein, the Amount of Acid Varying.

No. of cc. of acid used.	Strength of acid used.	Amount of acid used equal to N/100 HCl.	Preparation 1.		Preparation 2.		Preparation 3.		Preparation 4.	
			N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.	N/100 HCl in filtrate.	N/100 HCl in proteid.
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
30	N/200	15.0	8.8	6.2	9.2	5.8	8.6	6.4	8.8	6.2
20	N/100	20.0	13.6	6.4	13.2	6.8	13.4	6.6	13.6	6.4
20	N/80	25.0	19.2	5.8	19.2	5.8	19.0	6.0	19.6	5.4
10	N/60	16.6	10.8	5.8	10.6	6.0	10.8	5.8	11.0	5.6
	Average,	. .	. .	6.0	.	6.1	. .	6.2	. .	5.9

stand 1 hour with occasional agitation and were then filtered and aliquot portions of the filtrate titrated as in the preceding experiments. The results are given in Table III. :

Table III.—Amount of Hydrochloric Acid Neutralized by One Gram of Base-Free Casein, Amounts and Dilution of Acid Varying.

No. of cc. of acid used.	Strength of acid used.	Amount of acid used equal to N/100 HCl. cc.	Acid equal to N/100 HCl in filtrate. cc.	Acid equal to N/100 HCl in proteid. cc.
50	N/200	25.0	18.4	6.6
40	N/100	40.0	33.6	6.4
30	N/80	37.5	30.0	7.5
20	N/60	33.3	25.6	7.7

The results in Table III. indicate that, when we treat the base-free casein with a more concentrated acid solution, we have more acid left in the proteid. This is as we should expect under the conditions of experiment. The acid that is not recovered in the filtrate must either combine with the proteid or adhere to it mechanically. We made no attempt to wash the adherent or occluded acid from the proteid, because any thorough washing would remove some of the combined acid ; therefore, the more concentrated the acid used in treating the proteid, the more acid, as measured by titration, adhered to the proteid in addition to the fixed amount of acid that combined with it as compared with treatment with an equal volume of more dilute acid.

It is not easily conceivable that such an amount of acid as is represented by about 6 cc. of N/100 hydrochloric acid should be held only mechanically by 1 gram of casein, when we used solutions so dilute. Bearing on this point, the following additional experiments were made :

(1) We suspended a weighed amount of base-free casein in 50 cc. of water, allowed to stand 15 minutes, added N/100 hydrochloric acid at the rate of 6 cc. for 1 gram of casein and made the volume to 100 cc. with water. After filtration, the filtrate was titrated and the result showed that 5.2 cc. of N/100 hydrochloric acid had been held by 1 gram of casein.

(2) The experiment was repeated, allowing the mixture to

stand 1 hour after addition of acid. The result was the same as before.

(3) The experiment was repeated, except that we used only 4 cc. of N/100 hydrochloric acid for 1 gram of casein. The filtrate, on titrating, showed no acid, indicating that the entire 4 cc. of N/100 hydrochloric acid had been held by the casein.

These results indicate to us that 1 gram of base-free casein combines with about 0.5 cc. of N/10 hydrochloric acid and that the disappearance of acid is due to chemical union with proteid and not merely to mechanical mixture or adhesion.

The question has probably come to mind before this as to why free casein was not sooner isolated in the normal souring of milk, or in the treatment of milk by direct addition of acid; the cause can now readily be seen. Because it requires so little acid to change free casein into its acid combination, the point is quickly passed when we have any considerable proportion of free casein in the milk. In the normal souring of milk we were able to catch the change at a point where 65 per cent of the casein of the milk was in the form of free casein.[1] When we use an acid to precipitate casein from milk, an excess is added, so that we get, not the free casein, but the compound formed by its combination with acid.

Summary of Action of Acids on Calcium Casein (Milk-Casein).—When calcium casein is treated with an acid, the first reaction is a union between the acid and the calcium combined with casein, resulting in the formation of base-free casein, a compound insoluble in water, but soluble in warm 5 per cent solution of sodium chloride and in hot 50 per cent alcohol, a compound which, when warmed, also possesses the property of being very plastic and capable of being drawn out into long, fine, silky threads. This base-free casein is identical with the compound which we formerly regarded as being a casein monosalt of the acid used as precipitant.

When 1 gram of this base-free casein is treated with an amount of acid equivalent to about 0.5 cc. in the case of N/10 hydrochloric acid, the properties of the casein are changed, so that it is no longer soluble in warm 5 per cent salt solution and

[1] THIS JOURNAL, 32, 153 (1904).

only slightly soluble in hot 50 per cent alcohol; in addition it has lost entirely its plastic properties and the power of being drawn out into fine threads when warm. This substance we regard as resulting from the combination of acid with the base-free casein, forming a casein salt of the acid used. Our reasons for believing that there is actual chemical combination between the casein and acid are the following:

(1) When a given amount of base-free casein is treated with an acid, a quite definite and constant amount of acid appears to be neutralized. In the case of N/10 hydrochloric acid, 1 gram of base-free casein appears to combine with about 0.5 cc. of acid.

(2) The phenomenon of combination between proteids and acids appears to be very general, as shown by Cohnheim and others.[1]

(3) When base-free casein is treated with an acid it undergoes a marked change in its properties.

(4) Although the combination is comparatively weak, it appears to be as strong as in the case of some quite stable inorganic salts. The fact that the acid can be completely removed by long-continued trituration with water does not constitute an argument against combination, because some well-known inorganic salts behave in the same way under similar treatment.

(5) By treating base-free casein with a given amount of acid, we prepare the compound insoluble in warm dilute salt solution and hot alcohol. By removing the acid from this substance, we obtain again the base-free casein with a restoration of its recognized properties of solubility and plasticity. Each of these compounds can be converted into the other by addition or removal of acid.

The Relation of Casein and Its Compounds to Paracasein and Its Compounds.

The coagulum formed by calcium casein, when treated with rennet enzyme, was called "Käse" by Hammarsten.[2] Schulze and Rösc[3] suggested the name "paracasein" for this

[1] Chemie der Eiweisskörper, pp. 106–114, 2d Ed., (1904).

[2] Maly: Jahresb. d. Thierchemie, 4, 138 (1874).

[3] Landw. Versuchs-Stat., 31, 131 (1884-5).

product and their suggestion has been very generally accepted. There is still, however, much confusion as to the exact application of this term, as we shall point out later.

According to Hammarsten[1] and others, the action of rennet enzyme on calcium casein of milk takes place in two distinct stages: (1) The rennet enzyme converts the milk-casein into paracasein, but there is no coagulation or visible change, the paracasein apparently remaining in the same condition as the milk-casein. In the absence of soluble calcium salts, the paracasein remains in this uncoagulated condition in the case of normal milk. (2) Coagulation or separation of curd takes place in the presence of soluble calcium salts. The conversion of milk-casein into paracasein, in the first stage, is due to the rennet enzyme alone, while the coagulation of the paracasein, in the second stage, is due to soluble calcium salts alone, in the case of normal milk. The term paracasein is commonly applied to both the coagulated and uncoagulated forms.

Just what chemical change, if any, takes place in the proteid in the conversion of calcium casein into paracasein by rennet enzyme has not been clearly demonstrated. According to Hammarsten's original theory,[2] by the action of rennet enzyme, the calcium casein of the milk undergoes a hydrolytic separation into two compounds: (1) A body difficultly soluble, forming the chief product and closely resembling casein in composition—*paracasein*; (2) an easily soluble, albumose-like body, called by him *whey-proteid*, produced in very small amount. Further, the paracasein has not the property of holding calcium phosphate in solution to the same extent that casein has. Hammarsten later modified these views,[3] coming to the conclusion that the action of rennet enzyme rearranges the casein molecule only in a physical way. Du Claux[4] regards casein as existing in milk in three different forms, in equilibrium with one another; this equilibrium can be easily disturbed by the action of acids or ferments. Coagulation, according to his theory, is a purely physical, mechanical change. A similar conclusion is reached by Fuld,[5] milk coag-

[1] Maly: Jahresb. d. Thierchemie, **2**, 118 (1872); **7**, 158 (1877).
[2] *Ibid.*, **4**, 153 (1874).
[3] Z. physiol. Chem., **28**, 114 (1899).
[4] Compt. rend., **98**, 373.
[5] Beiträge z. Chem. Physiol. und Pathol., **2**, 169 (1902).

ulation being regarded as a special case of the alternation between suspension and precipitation of a colloidal substance. Eugling[1] concluded, from work done by him, that acids or rennet, when added to milk, render the insoluble calcium salts soluble and more readily available for the coagulation of the proteid. De Vries and Boekhout[2] believe that free acid exerts a direct influence on its own account upon the coagulation of paracasein, in some way independent of the formation of soluble calcium salts. Loevenhart[3] has stated that there is no chemical difference between casein and paracasein and that the observed differences in properties are due to physical changes, casein and paracasein being physical modifications of one and the same substance. According to his belief paracasein, in its solutions, exists in larger molecular aggregations than does casein.

We thus see that, while there is a wide range of opinion in regard to the relation of casein and paracasein, there is a prevailing tendency to regard these proteids, milk-casein and paracasein, as being essentially alike in composition.

It is our purpose to consider paracasein and some of its compounds in a similar manner to the discussion of casein given in the preceding pages; the topics will be taken up in the following order:

I. The relation of inorganic compounds to paracasein.

II. The relation of the salt-soluble compound of paracasein to paracasein.

III. The relation between the two series of compounds previously called paracasein mono-salts and paracasein di-salts.

IV. The relation of casein and its compounds to paracasein and its compounds.

(*I.*) *The Relation of Inorganic Compounds to Paracasein.*

In order to study paracasein in its relations to calcium and to acids, it was essential to obtain a paracasein that should be practically base-free. Preparations, made by coagulating milk with rennet and washing the coagulum, are high in ash content, containing often 4–5 per cent; moreover, such preparations still

[1] Landw. Versuchs-Stat., **31**, 392 (1885).
[2] *Ibid.*, **55**, 221 (1901).
[3] Z. physiol. Chem., **41**, 177 (1904).

contain the calcium originally combined in the calcium casein of the milk and are not satisfactory for such work as we desired to do. We therefore made a preparation of paracasein as follows:

Preparation of Paracasein.—To 1 liter of skim-milk we added 1.25 grams of ammonium oxalate; the milk, after standing a short time, was filtered through paper. To 500 cc. of this filtrate we added 0.05 cc. of Hansen's rennet extract and kept the whole at a temperature of 37°, for 2 hours. A small amount of thymol was added. To show that the rennet enzyme had acted upon the calcium casein in the milk, we took 10 cc. of the treated milk, cooled to room temperature and added 2 drops of 10 per cent solution of calcium chloride. In a few minutes the milk had formed a firm coagulum, which indicated that the rennet enzyme had produced paracasein. The 500 cc. of treated milk we now diluted with 16 liters of warm distilled water and added dilute hydrochloric acid until the proteid had separated. This precipitation was accompanied by vigorous stirring to keep the solid as finely divided as possible, thus facilitating the removal of inorganic salts. After the precipitate had settled, the supernatant liquid was removed, the precipitate was filtered and then triturated with warm water and filtered, the trituration and filtering being repeated until all trace of chlorides was removed, which required 4 days. The precipitate, after removal of acid, was soluble in warm 5 per cent solution of sodium chloride and in hot 50 per cent alcohol. Finally, the preparation was washed with strong alcohol and ether and dried at 100°. This treatment greatly lessened its solubility in dilute salt solution and alcohol. It was nearly ash-free, containing 0.11 per cent and gave no test for chlorides when boiled with nitric acid and silver nitrate.

Solubility of Paracasein Preparation in Lime-Water.—The paracasein, prepared in the manner described, was soluble in lime-water, forming a solution having a dull, opalescent appearance.

Preparation of Basic Calcium Paracasein.—We dissolved about 2 grams of our paracasein preparation in lime-water and made the solution neutral to phenolphthalein by the addition of

N/10 hydrochloric acid. The solution became more milky in appearance, but no precipitation was observable. This solution was now precipitated by alcohol, filtered, washed several times with alcohol, finally with ether and dried at 100°. The preparation was insoluble in dilute salt solution and practically so in hot dilute alcohol. One gram contained a total of 0.0283 gram of calcium oxide and 0.0063 gram of chlorine (equivalent to 0.0047 gram of calcium oxide combined as calcium chloride). Deducting the calcium oxide equivalent, present as chloride, from the total, we have 0.0236 gram, which represents the amount of calcium oxide combined with the proteid, or 2.36 per cent. This preparation, therefore, corresponds very closely in its calcium content with the basic calcium casein. As in the case of the calcium casein preparations, all those of calcium paracasein contained calcium chloride.

Preparation of Neutral Calcium Paracasein.—When we treat a lime-water solution of paracasein with an amount of acid sufficient to turn blue litmus red, or just a little short of this, the proteid separates as a precipitate and this occurs before all the calcium of the original lime-water solution has been neutralized. This product is insoluble in dilute salt solution and nearly insoluble in hot dilute alcohol; it was thrown on a filter and washed several times with water. Filtration was rather slow. The precipitate was then washed with alcohol and ether and dried at 100°. One gram contained 0.0391 gram of ash, a total lime content of 0.0194 gram of calcium oxide and 0.0064 gram of chlorine (equivalent to 0.0048 gram of calcium oxide combined as calcium chloride). Deducting the calcium oxide equivalent, present as chloride, from the total calcium oxide, we have 0.0146 gram, which represents the amount of calcium oxide combined with the proteid, or 1.46 per cent. This preparation, therefore, corresponds closely to the neutral calcium casein.

We have seen above that the neutral calcium paracasein, formed by making a lime-water solution of paracasein neutral to litmus, by acid, separates as a precipitate, while neutral calcium casein does not separate thus. At the point of neutrality with litmus, the body formed, that is, neutral calcium

paracasein, is precipitated. *In this respect and in this respect only, does there appear to be a marked difference between the properties of casein and paracasein compounds.* This is in harmony with what takes place when rennet acts on milk, in the presence of soluble calcium salts—an insoluble calcium paracasein is formed. It shows that our preparation of paracasein had not reverted to casein.

Preparation of Base-Free Paracasein.—In 50 cc. of lime-water, requiring 21.1 cc. of N/10 hydrochloric acid for neutralization, we dissolved 0.5 gram of our ash-free paracasein. The solution became neutral to phenolphthalein after the addition of 16.9 cc. of N/10 hydrochloric acid. This leaves calcium oxide equivalent to 4.2 cc. of N/10 hydrochloric acid as combined with the proteid, or 0.01176 gram of calcium oxide, which is 2.35 per cent of the paracasein and this is the basic calcium paracasein.

When 18 cc. of N/10 hydrochloric acid had been added a precipitate began to form, but the solution was still alkaline to litmus. When 18.5 cc. of the acid had been added, precipitation was complete. This leaves calcium oxide equivalent to 2.6 cc. of N/10 hydrochloric acid as combined with the proteid, or 0.00728 gram of calcium oxide, which is 1.46 per cent of the paracasein, and this is the neutral calcium paracasein.

When 21.1 cc. of N/10 hydrochloric acid had been employed, an amount necessary to neutralize exactly the lime-water used for dissolving the paracasein, the proteid was still precipitated. It was now quite easily soluble in warm 5 per cent salt solution and nearly so in hot 50 per cent alcohol. On warming, the precipitate settled on the bottom of the beaker and was easily gathered in a mass, on a stirring rod. This mass was very plastic, showing a tendency to flow when kept warm; it also possessed the property of being drawn into long, fine, silky threads. It behaved in every respect like *base-free casein.*

In another experiment we dissolved 1.5 grams of our paracasein preparation in lime-water and then exactly neutralized the solution with N/10 hydrochloric acid. The precipitated proteid was washed with alcohol and ether and dried at 100°. Determinations of calcium and chlorine showed their presence

in the proportions found in calcium chloride, which was probably held in the proteid mass mechanically.

Behavior of Lime-Water Solutions of Paracasein Compounds toward Rennet and Soluble Calcium Salts.—Basic calcium paracasein, prepared by dissolving base-free paracasein in lime-water and making neutral to phenolphthalein, is not coagulated by rennet in the presence or absence of soluble calcium salts. However, a solution of basic calcium paracasein may be coagulated on warming, by soluble calcium salts alone, without rennet.

Neutral calcium casein, prepared by dissolving base-free paracasein in lime-water and making neutral to litmus, is coagulated readily at room temperature, or on warming, by soluble calcium salts, with or without rennet, but not in the absence of soluble calcium salts.

Rennet changes calcium casein to calcium paracasein, but does not coagulate the proteid. Soluble calcium salts coagulate neutral calcium paracasein and the action takes place through quite a wide range of temperature, while basic calcium paracasein is coagulated by soluble calcium salts only after warming. Here, as in the case of the corresponding calcium casein compounds, the coagulation may be the result of purely physical change, or there may be a loose combination between the soluble calcium salt and the calcium paracasein compound.

Summary of Results of Work Done on Paracasein and Its Compounds.—We have made and studied the following paracasein preparations: (1) Base-free paracasein, the free proteid; (2) basic calcium paracasein, containing about 2.40 per cent of calcium oxide; (3) neutral calcium paracasein, containing about 1.50 per cent of calcium oxide.

(*II.*) *The Relation of the Salt-Soluble Compound of Paracasein to Paracasein.*

Of the three paracasein preparations made and studied by us, we have found only one that was readily soluble in warm 5 per cent salt solution and hot 50 per cent alcohol and this was the base-free paracasein. This body, on warming, showed the peculiar plastic property and the power of being drawn into

long, fine, silky threads, which are shown by the salt-soluble substance, prepared by us from cheese-curd and cheese. This is the body which our former work led us to regard as a compound formed by combination of paracasein and lactic acid, in the case of cheese and which we regarded as paracasein monolactate. As the result of our more recent work, we believe that the compound formed by treating calcium paracasein (cheese-curd) with an amount of acid just sufficient to combine with the calcium of the calcium paracasein, in addition to certain inorganic salts held mechanically in the cheese-curd, is not paracasein monolactate but base-free paracasein, or calcium paracasein, from which calcium has been removed by its combination with acid.

(*III.*) *The Relation between the Two Series of Compounds Previously Called Paracasein Mono-Salts and Paracasein Di-Salts.*

When to base-free paracasein we add dilute acid, another body appears to be formed, which differs in properties from the base-free proteid in being insoluble in warm 5 per cent salt solution and hot 50 per cent alcohol and also in possessing none of the peculiar plastic and ductile properties of the base-free paracasein. This substance is formed when milk is treated with rennet enzyme and allowed to coagulate, either by spontaneous souring or by direct addition of dilute acids. We at first regarded the compound as a paracasein di-salt of an acid, but since we have shown that what we regarded as a paracasein mono-salt is the base-free proteid, paracasein, we now regard this compound as resulting from the combination of acid with the base-free paracasein, forming a paracasein salt of the acid used, corresponding to the casein salts of acids, which have been already discussed.

In each of several experiments we suspended, in distilled water, portions of 0.5 gram of base-free paracasein and treated this with varying amounts of dilute acid of different strengths, agitating from time to time, for 1 hour. The mixture was filtered and the filtrate titrated with N/100 hydrochloric acid. The amount of acid not recovered in the filtrate is regarded as representing, approximately, the quantity that had combined

with the paracasein. This was found to be equivalent to about 0.5 cc. of N/10 hydrochloric acid, closely agreeing with the results obtained by treating base-free casein with acid.

(*IV.*) *The Relation of Casein and Its Compounds to Paracasein and Its Compounds.*

We have made preparations of the following compounds, which have been described in the preceding pages:

Casein, the base-free or uncombined proteid.

Basic calcium casein, containing the free proteid combined with about 2.40 per cent of calcium oxide.

Neutral calcium casein, containing the free proteid combined with about 1.50 per cent of calcium oxide, probably identical with casein as it exists in cows' milk.

Casein salts of acids, formed by combination of the free proteid with acids.

Paracasein, the base-free or uncombined proteid.

Basic calcium paracasein, free paracasein combined with about 2.40 per cent of calcium oxide.

Neutral calcium paracasein, free paracasein combined with about 1.50 per cent of calcium oxide, probably identical with the uncoagulated body present in cows' milk treated with rennet enzyme, in the absence of soluble calcium salts.

Paracasein salts of acids, formed by combination of the free proteid with acids.

A comparison of the properties of casein and paracasein and their compounds strongly suggests that they are chemically alike, paracasein and its combinations being larger molecular aggregations than casein and its corresponding compounds, in accordance with the suggestion of Loevenhart. The following statements serve to bring out the close resemblance more strikingly:

(1) The free proteids, casein and paracasein, possess the same solubilities in warm 5 per cent salt solution and hot 50 per cent alcohol. They possess the same peculiar plastic and ductile properties when warmed.

(2) Casein and paracasein form compounds containing the same amounts of combined calcium oxide.

(3) Casein and paracasein, when treated with dilute acid in

the proportion of 1 gram to about 0.5 cc. of N/10 hydrochloric acid, are changed into bodies having the same properties, which differ strikingly from those of the free proteids.

(4) Neither basic calcium casein nor basic calcium paracasein is coagulated by rennet, either in the presence or absence of soluble calcium salts. Both these compounds are coagulated when warmed to 35°–45° in the presence of soluble calcium salts, but not in the absence of such salts.

(5) Neutral calcium casein (present in milk) is coagulated by a few drops of a soluble calcium salt on warming and neutral calcium paracasein (present in milk acted upon by rennet enzyme) is coagulated, at ordinary temperatures, by soluble calcium salts. Neither neutral calcium casein nor neutral calcium paracasein is coagulated by rennet in the absence of soluble calcium salts.

(6) Neutral calcium casein, prepared by making a lime-water solution of free casein neutral to litmus, is an opalescent solution, free from any visible suspended particles; neutral calcium paracasein, prepared by making a lime-water solution of free paracasein neutral to litmus, is a clearly defined coagulum. In this respect only does there appear to be any marked difference in the behavior of the neutral calcium compounds of casein and paracasein. Here the difference is one of degree rather than of kind, because neutral calcium casein is coagulated by small amounts of soluble calcium salts on warming, while neutral calcium paracasein is coagulated at lower temperatures by soluble calcium salts.

The Relation of the Salt-Soluble, Alcohol-Soluble Product in Cheese to Paracasein.

It was from our work with cheese (American cheddar) that we gained the first suggestion which led us to investigate this field. It is desirable, therefore, that we should now, if possible, establish the relation between the salt-soluble, alcohol-soluble body obtained from cheese, which we were first led to regard as paracasein monolactate and the paracasein compounds we have prepared from milk and discussed in the preceding pages.

We have established the fact that fresh cheddar cheese con-

tains a large percentage of its proteid in the form of a body soluble in warm 5 per cent salt solution and in hot 50 per cent alcohol. Weidmann[1] dissolved, from Emmenthaler cheese, by hot alcohol, a substance called by him caseoglutin. Röse and Schulze[2] made a quite extensive study of the properties of this compound but reached no definite conclusions as to whether it was a chemical individual or what was its relation to cheese-ripening. Winterstein[3] has studied the cleavage-products of caseoglutin treated by concentrated hydrochloric acid and finds the proteid bases arginine and lysine present, in proportions differing from those obtained by Hart from casein. The results of his work cannot be regarded as conclusive, because variation of conditions in producing cleavage-products leads to quite variable results. A cleavage study made by us[4] on a similar product suggests a close relation between casein and the salt-soluble substance, which appears to be identical with caseoglutin.

Material Used to Furnish the Preparation.—We used, as the source of our material, a cheddar cheese 1 day old, which indicated, by forming strings on a hot iron, an abundance of salt-soluble substance. Portions of the cheese were ground in sand and extracted with water at 55°. The portion insoluble in water was then divided, part to be extracted with hot 50 per cent alcohol and part with warm 5 per cent salt solution.

Preparation Made by Extraction with Alcohol.—The ground, water-extracted cheddar-curd was treated with boiling 50 per cent alcohol. The extract was filtered through paper, on a hot-water funnel. The filtrate, on cooling and standing, deposited the extracted proteid in a rubber-like mass. This was ground up in water several times, filtered, washed with 95 per cent alcohol and ether, and dried at 100°. The product was a hard mass, difficult to pulverize. It contained 1.40 per cent of ash.

Preparation Made by Extraction with 5 Per Cent Solution of Sodium Chloride.—The remaining portion of ground, water-

[1] Landw. Jahres., 1882, p. 587.
[2] Landw. Versuchs Stat., 31, 130 (1884–5).
[3] Z. physiol. Chem., 41, 487 (1904).
[4] This Journal, 28, 420 (1902).

extracted cheese was extracted with a 5 per cent solution of sodium chloride, at 60°, filtered through paper twice and then precipitated with 2 volumes of 95 per cent alcohol. On standing over night the precipitate separated. It was filtered, washed by decantation with water several times to remove the salt and finally thrown on a filter-paper, washed with alcohol and ether and dried at 100°. The product was easily powdered. It contained 1.37 per cent of ash.

Behavior of Salt-Soluble and Alcohol-Soluble Preparations toward Lime-Water.

We made a study of the power of these two preparations to combine with calcium for comparison with base-free casein, in order to ascertain whether they were base-free or not. In portions of 50 cc. of lime-water, of known strength, we dissolved 0.5 gram each of base-free casein, of the salt-soluble preparation and of the alcohol-soluble preparation. These lime-water solutions of proteids were then treated with N/10 hydrochloric acid until the solution was neutral to phenolphthalein, with the following results:

Table IV.—Power of Different Preparations to Combine with Calcium.

	N/100 HCl required to neutralize.	Difference of N/10 HCl between lime-water and solutions of proteids in lime-water.	Equivalent to CaO combined with proteid.	Per cent of CaO combined with proteid.
	cc.	cc.	Gram.	
50 cc lime-water	19.5	.	. .	. .
0.5 gram of base-free casein, dissolved in 50 cc. lime-water	15.5	4.0	0.0112	2.24
0.5 gram of alcohol preparation, dissolved in 50 cc. lime-water	15.4	4.1	0.01148	2.29
0.5 gram of salt-soluble preparation, dissolved in 50 cc. lime-water	15.6	3.9	0.01093	2.19

These results show that the salt-soluble and alcohol-soluble preparations, made from cheese, possessed the same power of

combining with calcium as do casein or paracasein known to be base-free and that they must, therefore, have been base-free proteids, capable of forming a calcium combination equivalent to that of basic calcium casein or paracasein. These preparations from cheese appear, therefore, to be identical with base-free paracasein.

The calcium of the ash present was evidently not a part of the proteid molecule, but was probably an impurity of calcium lactate, or possibly this salt loosely combined with the proteid, appearing, on ignition, as calcium phosphate.

Behavior of Salt-Soluble and Alcohol-Soluble Preparations toward Acids.

In determining the amount of acid that could combine with these salt-soluble and alcohol-soluble preparations made from cheese, we used the methods already employed in connection with casein and paracasein. We suspended 0.5 gram of proteid in water, added 10 cc. of N/100 hydrochloric acid, made the volume up to 100 cc., filtered and titrated the filtrate with N/100 sodium hydroxide, with phenolphthalein as indicator. The results, calculated on the basis of 1 gram of proteid, were as follows:

Table V.—Acid-Combining Power of Different Preparations.

Material used.	Amount of acid used equal to N/100 HCl. cc.	Acid equal to N/100 HCl in filtrate. cc.	Acid equal to N/100 HCl in proteid. cc.
Base-free casein	20	13.6	6.4
Alcohol-soluble preparation	20	13.6	6.4
Salt-soluble preparation	20	14.0	6.0

In respect to acid-combining power, these results indicate that the alcohol-soluble and salt-soluble preparations made from cheese are essentially the same compounds as casein and paracasein, that is, they are essentially base-free paracasein.

The Nomenclature of Casein and Paracasein and Their Compounds.

From the work presented in the preceding pages there is reason to believe that there exist the following bodies, repre-

senting milk-casein and paracasein and the compounds formed by them:

Casein, basic calcium casein, neutral calcium casein, casein salts of acids, paracasein, basic calcium paracasein, neutral calcium paracasein and paracasein salts of acids.

In most of the literature on the subject, the word casein is used indiscriminately to mean milk-casein, free casein, or those casein salts formed by acid precipitation. In many cases it is used comprehensively to include all the proteids in cows' milk. A similar state of confusion exists in regard to the use of the word paracasein. It would, therefore, seem pertinent to make the following suggestions, tentatively at least, in regard to the nomenclature of these compounds:

(1) That the compound existing in cows' milk, commonly called casein, be called calcium casein.

(2) That the word casein be applied only to the free proteid, that is, the base-free casein.

(3) That the casein compound containing about 2.40 per cent of calcium oxide be called basic calcium casein.

(4) That a compound formed by precipitation and combination with an acid be called a casein salt of the acid used.

(5) That the same nomenclature be applied to the corresponding paracasein bodies, simply substituting the word paracasein for casein, with the following addition: The name calcium paracasein should be applied to the uncoagulated substance produced in milk by rennet enzyme, while the coagulum of this substance, caused by soluble calcium salts, should be called *coagulated* calcium paracasein.

The Relation of the Results to Certain Changes Taking Place during the Cheese-Making Process.

In the manufacture of cheddar and similar types of cheese, after the addition of rennet enzyme and coagulation, there takes place a progressive change, resulting in the production of increased amounts of a proteid soluble in warm 5 per cent solution of sodium chloride. This product may amount, in fresh cheese, to 75–80 per cent of the proteids present. We were led by the former work to interpret these facts as follows: Lactic acid, formed by the lactic fermentation of milk-

sugar, combines with the paracasein of the curd, forming paracasein monolactate, insoluble in water but soluble in warm 5 per cent salt solution and in hot 50 per cent alcohol. In the light of the results of our more recent work, this interpretation must be modified and the observed facts appear to be explained correctly in the following manner: The coagulum following the addition of rennet enzyme to milk is calcium paracasein, either mixed or loosely combined with soluble calcium salts. While lactic acid is being formed in the cheese-making process, it combines with the calcium of the calcium paracasein, forming free paracasein and calcium lactate. The conditions of manufacture are so controlled that, normally, not enough is produced to convert all the calcium paracasein to base-free paracasein. The proteids of the curd are, therefore, a mixture, in varying proportions, of calcium paracasein and free paracasein. It is the free paracasein that is soluble in warm 5 per cent salt solution and in hot 50 per cent alcohol; and it is this body that has the characteristic property of being drawn out in fine, silky threads, when touched with a hot iron. It is the free paracasein that imparts to cheese-curd its peculiar plastic and ductile properties, exhibited in the process known as "packing" or "matting." It is the free paracasein, therefore, that appears to be the substance in which the various chemical changes grouped under the general term of cheese-ripening begin to take place.

When, in the process of cheese manufacture, an excess of lactic acid is produced, 0.7–0.8 per cent, we have the product familiarly known as cottage or Dutch cheese. It has a loose, granular structure and is insoluble in warm 5 per cent salt solution. In this case all the calcium of the calcium paracasein combines with lactic acid, after which additional amounts of free lactic acid formed unite, in a loose combination, with the free paracasein, producing paracasein lactate, which differs from free paracasein in a most marked manner in respect to its solubilities in dilute salt solution and hot alcohol and in its plasticity and ductility.

Summary.

1. *Object.*—The primary object of the work presented is to

make a further study of the casein and paracasein salts of acids,[1] with an extension to the free proteids and some of their combinations with bases.

2. *Base-free Casein.*—Preparations of casein free from ash, or nearly so, were made by precipitating dilute skim-milk with acids, removing the acid and inorganic matter by repeated filtration and trituration with water in a mortar. The process required several days.

3. *Basic Calcium Casein.*—Compounds were made of base-free casein in which the proteid combined with about 2.40 per cent of calcium oxide. One such preparation was made by triturating together calcium carbonate and the base-free casein, suspended in water and another by dissolving base-free casein in lime-water and making this neutral to phenolphthalein by acid.

4. *Neutral Calcium Casein.*—By treating base-free casein, dissolved in lime-water, with acid until the liquid is almost neutral to litmus, there is formed a compound of casein and calcium oxide containing about 1.50 per cent of calcium oxide.

5. *Calcium Casein Compounds in Relation to Rennet Enzyme and Soluble Calcium Salts.*—Rennet enzyme coagulates neither neutral nor basic calcium casein. Neutral calcium casein, after treatment with rennet, is coagulated at ordinary temperatures by soluble calcium salts. Soluble calcium salts, such as calcium chloride, coagulate both neutral and basic calcium casein on warming to 35°–45°.

6. *Casein Present in Milk as Neutral Calcium Casein.*—In its behavior toward soluble calcium salts on warming and at ordinary temperatures, after treatment with rennet, neutral calcium casein behaves like milk-casein and casein is probably present in cows' milk as the neutral calcium casein.

7. *Identity of Base-Free Casein and the Salt-Soluble Proteid.*—A base-free casein, prepared either directly from milk or by treating a lime-water solution of free casein with an acid to the point of acidity with litmus, is readily soluble in warm 5 per cent salt solution and in hot 50 per cent alcohol. This body, when freshly prepared and sufficiently warmed, is very plastic

[1] THIS JOURNAL, 28, 411 (1902).

and ductile. It behaves, in all respects, like the substance which we were formerly led to regard as a compound formed by combination of casein and an acid and which we regarded as a casein mono-salt of the acid precipitant.

8. *Relation between the Two Series of Compounds Previously Called Casein Mono-Salts and Casein Di-Salts of Acids.*—When 1 gram of base-free casein is treated with about 0.5 cc. of N/10 hydrochloric acid, a substance is formed which is insoluble in warm 5 per cent salt solution and in hot 50 per cent alcohol and which is no longer plastic or ductile on warming. This is like the substance usually formed when milk coagulates by natural souring. By treating base-free casein with dilute acid, it was found that 1 gram of base-free casein appears to combine with about 0.5 cc. of N/10 hydrochloric acid, forming a casein salt of hydrochloric acid. While the compounds formerly regarded by us as casein mono-salts of acids have been shown by us to be identical with base-free casein, the compounds which we called casein di-salts of acids are compounds formed by combination of acids with free casein.

9. *Paracasein and Its Compounds.*—A preparation of base-free paracasein was made and from this, dissolved in lime-water, were prepared (1) basic calcium paracasein, containing in combination about 2.40 per cent of calcium oxide, (2) neutral calcium paracasein, containing about 1.50 per cent of combined calcium oxide.

10. *Comparison of Properties of Casein and Paracasein and Their Compounds of Calcium.*—Basic calcium casein and paracasein appear to be soluble in water, forming slightly opalescent solutions. Neither is coagulated by rennet, but both are precipitated by soluble calcium salts on warming. Neutral calcium casein is coagulated by soluble calcium salts on warming to 35°-45°, but not at ordinary room temperatures, while neutral calcium paracasein is completely and quickly coagulated, at room temperatures, by soluble calcium salts. Free casein and free paracasein, freshly prepared, possess the same solubilities in warm 5 per cent salt solution and in hot 50 per cent alcohol; they also possess the same characteristic properties of plasticity and ductility. The close resemblance of casein and

its compounds to paracasein and its compounds, respectively, suggests that they are chemically alike, paracasein being different only by consisting of a larger molecular aggregation than casein.

11. *Relation of Paracasein to Salt-Soluble Substances and to Compounds Formed by Treatment with Acid.*—Free paracasein appears to be identical in characteristic properties with the compounds we formerly called paracasein mono-salts of the acids used as precipitants. The compounds which we have heretofore called paracasein di-salts of acids appear to be combinations of free paracasein and the acids used as precipitants, 1 gram of paracasein uniting, for example, with about 0.5 cc. of N/10 hydrochloric acid.

12. *Relation of the Salt-Soluble Product of Cheese to Paracasein.*—From water-extracted, fresh cheddar cheese we prepared one extract by warm 5 per cent salt solution and another by hot 50 per cent alcohol. These preparations have, in common with free paracasein, the characteristic properties of plasticity and ductility and the same combining power with bases and acids, they therefore appear to be free paracasein instead of paracasein monolactate, as we were formerly led to believe.

13. *Chemical Changes in Calcium Casein Resulting from Souring of Milk or Addition of Acids.*—When an acid is formed in cows' milk, or added to it, the acid first combines with the bases of some of the inorganic salts of the milk and then with the calcium that is combined with the casein, resulting in the formation of a precipitate which is free casein. By further formation, or addition of acid, the free casein unites with it, forming a casein salt of the acid, this compound, in the case of lactic acid, being the coagulum familiar in the ordinary souring of milk.

14. *Chemical Changes in Calcium Paracasein during the Process of Making Cheddar Cheese.*—The coagulum, following the addition of rennet enzyme to milk, is calcium paracasein, either mixed or loosely combined with soluble calcium salts. While lactic acid is being formed in the process of cheese-making, it combines with the calcium of the calcium paracasein, forming free paracasein and calcium lactate. It is this

free paracasein, thus formed, that is soluble in warm dilute salt solution and in hot 50 per cent alcohol and possesses characteristic properties of plasticity and ductility.

15. *Suggestions in Regard to the Nomenclature of Casein and Paracasein and Their Compounds.*—Much confusion prevails, at present, in the use of the terms casein and paracasein. It is suggested that the following nomenclature be used: (1) That the compound existing in cows' milk be called calcium casein. (2) That only the free proteid be called casein. (3) That the casein compound containing 2.40 per cent of calcium oxide be called basic calcium casein. (4) That a compound formed by precipitation and combination with acid be called a casein salt of the acid used. (5) That the same nomenclature be applied to the corresponding paracasein bodies, with the following addition: Calcium paracasein should be applied to the uncoagulated form and the term *coagulated* calcium paracasein to the coagulated form.

GENEVA, N. Y.,
Dec., 1904.

Contributions from the Chemical Laboratory of Harvard College.

NOTE ON THE PREPARATION OF CERTAIN AMINES.

BY LATHAM CLARKE.

In the course of a research[1] on addition compounds formed from dimethylaniline and allied substances, it was necessary to have moderately large quantities of several dialkylated amines. In preparing these I have worked out a process which is more convenient than the older methods and gives a much larger yield; for example, this method gave, with *o*-toluidine and methyl iodide, a practically quantitative yield of dimethylorthotoluidine and with *p*-toluidine and methyl iodide gave 76–79 per cent of the theoretical yield of dimethylparatoluidine. By Thomsen's method,[2] on which mine was founded, not over 30 per cent was obtained in any case.

I have also succeeded in improving the method for making benzylamine, so that, in a single case, from 100 grams of

[1] Jackson and Clarke: Ber. d. chem. Ges., 37, 176.
[2] Ber. d. chem. Ges., 10, 1586.

benzyl chloride I obtained 40 grams of this substance instead of the 25 grams yielded by Mason's[1] original process.

The dimethyltoluidines have been prepared by two methods, Thomsen's[2] and Nölting's.[3] Thomsen's method consists in treating the amine repeatedly with methyl iodide and caustic soda, forming the trimethyltolylammonium iodide and this, by treating with lead oxide, yields the hydrate, which is subjected to distillation, several steps thus being involved, the last a somewhat wasteful one. Nölting seals up the amine in a tube with methyl alcohol and hydrochloric acid, heats the mixture for 2 days at 200°–220° and then rectifies the product. Both these methods are somewhat tedious and the yields are not especially good.

It is best to illustrate my general method by a specific experiment. Ten grams of *p*-toluidine were treated with 75 grams of methyl iodide (approximately 5 times the necessary amount) in a round-bottomed flask, with a very long return-condenser attached. This was placed on a water-bath, at 50°, and the violent boiling which soon began was allowed to continue 30–40 minutes, shaking occasionally to prevent caking. The excess of methyl iodide was then distilled over the water-bath and, when practically all had been driven off, the white pasty residue was treated with an equal volume of water, which dissolved all but the small amount of methyl iodide left; by warming the liquid and passing a stream of air through it this was soon removed entirely. It is almost impossible to get out all the iodide by simple heating, but the success of the method depends on its complete removal. To the clear, somewhat colored liquid remaining a strong solution of potassic hydrate was then added and the floating, oily layer separated and dried with solid caustic potash.

After drying, the oil was subjected to another treatment with methyl iodide under the same conditions as before, the boiling being continued rather longer. The excess of iodide was distilled off, the residue dissolved in water and the last traces of iodide expelled as before. On treating with alkali

[1] J. Chem. Soc., 63, 1313.
[2] Ber. d. chem. Ges., 10, 1586.
[3] *Ibid.*, 11, 2279.

this time, the oil which separated generally sank to the bottom and was removed and dried over solid caustic potash. It was then distilled, when pure dimethyl paratoluidine passed over as a clear, colorless liquid, boiling at 183° (760 mm.). The yield was 10 grams, or 79.3 per cent of the theoretical.

The purity of the dimethylorthotoluidine and dimethylparatoluidine, obtained in this way, was proved by their constant boiling-points, halogen tests, which gave satisfactorily negative results and determinations of nitrogen (Dumas):

0.2345 gram ortho gave 22.0 cc. N at 24° and 760 mm.

	Calculated for $C_9H_{13}N$.	Found.
N	10.37	10.51

0.3511 gram para gave 33.2 cc. N at 24° and 760 mm.

	Calculated for $C_9H_{13}N$.	Found.
N	10.37	10.60

This method has several obvious advantages over Thomsen's or Nölting's. The excess of iodide has so kept down the temperature that there is no elimination of hydriodic acid before the treatment with alkali; before the alkali is added all methyl iodide is removed, thus preventing the formation of any trimethyltolylammonium iodide as a by-product. The great excess of methyl iodide also prevents any toluidine or methyltoluidine from remaining unchanged and the process is not wasteful, because almost all the excess can be saved and used again. The reaction is, therefore, made almost an exact one.

Using isoamylamine, methylisoamylamine and dimethylisoamylamine were prepared by this method, both for the first time. Methylisoamylamine is a clear, colorless liquid, with a peculiar, not unpleasant odor, it boils at 95° (758 mm.). From this the dimethylisoamylamine was made, which is also a clear, colorless liquid with a strong odor, boils at 98° (758 mm.), it gave the right percentage of nitrogen (Dumas), but, unfortunately, the record of the analysis is lost. In their preparation the reactions with methyl iodide are very violent and it was found best to use about 10 times the calculated

amount of the latter in each case. The temperature at which the excess of methyl iodide was driven off was not allowed to exceed 50°. Although the reaction proceeds well, yet lack of experience and the small quantities worked with caused the yields to be not very good, only about 40 per cent of the theoretical amount of dimethylisoamylamine being obtained.

Dimethylbenzylamine was prepared by this method very easily; it has been previously made by Jackson and Wing,[1] by the action of dimethylamine on benzyl chloride. The benzyl amine used in this work was obtained by a modification of Mason's method,[2] which proved successful the first and only time it was tried. Mason used a 15 per cent solution of ammonia in absolute alcohol and so much benzyl chloride that, approximately, 20 molecules of ammonia were present to 1 of benzyl chloride. This was allowed to stand 5 days and then the separation of the primary, secondary and tertiary amines formed in the reaction was proceeded with. In this manner 100 grams of benzyl chloride gave 25 grams benzylamine, 30 grams dibenzylamine and 6 grams tribenzylamine.

Like Mason, I used a practically saturated ammonia solution in absolute alcohol and the same proportion of ammonia to benzyl chloride, but in my case the benzyl chloride was dropped slowly from a separating-funnel and a Witt stirrer was made use of in such a way that the drops, as they entered, were violently torn apart and stirred deeply in, thus preventing the outer part of the drop, which had already reacted with the ammonia, from reacting with the inner part, which had not. The dropping was continued for 6 hours and the whole was then allowed to stand 3 days, when the amines were separated according to Mason's method. From 100 grams of benzyl chloride, 40 grams of benzylamine, 16 grams of di- and 4 grams of tribenzylamine were obtained.

From benzylamine a 40 per cent yield of dimethylbenzylamine was obtained which boiled at 185° (760 mm.) The methylbenzylamine was isolated and boiled at 175° (760 mm.). The reaction here with the methyl iodide is very violent, like

[1] THIS JOURNAL, 9, 79 (1887).
[2] J. Chem. Soc., 63, 1313.

that with isoamylamine and similar proportions of amine to methyl iodide were used.

α- and β-naphthylamines do not add methyl iodide readily enough to make this method applicable. When α- and β-dimethylnaphthylamines were desired they were obtained by the methods of Friedländer and Holman[1] and Hantzsch,[2] respectively.

Contributions from the Chemical Laboratory of Harvard College.

THE DETERMINATION OF OXALIC ACID BY PERMANGANATE IN THE PRESENCE OF HYDROCHLORIC ACID.

By Gregory Paul Baxter and Joaquin Enrique Zanetti.

The use of potassium permanganate as an oxidizing agent, in the presence of hydrochloric acid, has been the subject of frequent investigations which have made it certain that, in the oxidation of ferrous iron, there is invariably a loss of chlorine from the liquid unless manganous salts, phosphoric acid, or both are added to the ferrous solution, before titration. The determination of oxalic acid by permanganate, in the presence of hydrochloric acid, has received less attention. Zimmermann[3] concluded that hydrochloric acid does not interfere with the titration, but upon the basis of analyses where only 20 cc. of permanganate were employed and the volume determined only to tenths of a cc. Fleischer[4] makes the same statement. The various reference books upon quantitative analysis almost universally prescribe the addition of manganous salts to prevent loss of chlorine. Ostwald,[5] on the other hand, states that oxalic acid may be titrated with perfect sharpness, in the presence of hydrochloric acid, without a trace of chlorine being set free. Finally, Gooch and Peters[6] have shown conclusively that the process is an accurate one if a manganous salt is added

[1] Ber. d. chem. Ges., **21**, 3124 (1888).
[2] *Ibid.*, **13**, 1348.
[3] Ann. Chem. (Liebig), **213**, 305 (1882).
[4] Vol. Anal., Trans. Muir, 1877, p. 71.
[5] Foundations of Analyt. Chem., p. 151.
[6] Am. J. Sci., **7**, 461 (1899).

previously to titration, but their results, in cases where manganous salts were not employed, show a positive error in the quantity of permanganate necessary to complete the oxidation. However, in their experiments, the hydrochloric acid was added to solutions which already contained 10 cc. of approximately 50 per cent sulphuric acid, so that the acid concentration was much higher than that which usually exists in an analysis.

A study of the mechanism of the reaction by which chlorine is liberated by the permanganate, leads to the conclusion that the initial factors are the permanganate, hydrogen and chlorine ions, which unite in proportions, by numbers, indicated by one of the two possible equations:

$$MnO_4^- + 8H^+ + 5Cl^- \rightleftarrows Mn^{++} + 4H_2O + 5Cl;$$
$$2MnO_4^- + 11H^+ + 5Cl^- \rightleftarrows 2Mn^{++} + 5HOCl + 3H_2O.$$

According to either of these equations the reaction should be most susceptible to variations in the concentration of the hydrogen ion, somewhat less so to the concentration of the chlorine ion and least to that of the permanganate ion. If, then, the concentration of the hydrochloric acid is reduced to as low a value as is compatible with the rapid oxidation of the oxalic acid, it should be possible at least to minimize the loss of chlorine. The first experiments were directed to test this point.

A solution of potassium permanganate was prepared, containing about 4 grams in a liter. A solution of recrystallized oxalic acid contained about 12 grams per liter. First the strength of the permanganate was very carefully determined, by titration against 25 cc. portions of the oxalic acid solution, in the presence of sulphuric acid. After the relation between the permanganate and oxalic acid solutions had been accurately established, titrations were performed with the use of 10 cc. of dilute hydrochloric acid (sp. gr. 1.04++) for each 25 cc. portion of the oxalic acid, which was diluted to 150 cc. before titration. It was soon found that the volume of permanganate necessary depended upon the *temperature of the solution* during the titration. If the temperature was at or above 70° at the beginning of the titration, decolorization began as soon as a few cc. of the permanganate had been added and the results agreed exactly with those obtained by the use of sulphuric

acid instead of hydrochloric acid. On the other hand, if the temperature of the oxalic acid solution was below 70°, it was necessary to add more permanganate before the reaction visibly began and more permanganate was necessary to complete the oxidation. Furthermore, the solution had the peculiar odor of hypochlorous acid,[1] in all cases where more than the theoretical amount of permanganate was necessary. This hypochlorous acid was apparent only before the decolorization commenced and while it was proceeding. Beyond this point, *i. e.*, as soon as the decolorization of the permanganate took place as fast as the solution was introduced, no odor of hypochlorous acid or chlorine could be detected. Whenever the hypochlorous acid was produced, 15–30 cc. of the permanganate and at the lowest temperatures even more, were necessary for the first signs of reaction. The end-point was sharp in all cases. In the following table are given the results of the first series of experiments:

Table I.

$H_2C_2O_4$. cc.	HCl. cc.	Volume. cc.	Temperature at beginning.	$KMnO_4$ used. cc.	Theoretical $KMnO_4$. cc.	Error in per cent.
25	10	150	80° ±	45.72	45.74	—0.04
"	"	"	80° ±	45.74	"	0.00
"	"	"	80° ±	45.73	"	—0.02
"	"	"	80° ±	45.74	"	0.00
"	"	"	70°	45.74	"	0.00
"	"	"	70°	45.77	"	+0.07
"	"	"	65°	45.79	"	+0.11
"	"	"	60°	45.78	"	+0.09
"	"	"	50°	45.82	"	+0.18
"	"	"	40°	45.97	"	+0.50

It is probable that the action of the permanganate upon the hydrochloric acid, at low temperatures, produces, primarily, hypochlorous acid, which is slightly dissociated and readily volatile. That no appreciable amount of hypochlorous acid is formed at high temperatures may be due to more rapid removal, under these conditions, of either the permanganate or the hypochlorous acid by reaction with some other substance. The removal of the former may be due to the fact that potassium permanganate reacts more vigorously at high than at low

[1] If a solution of potassium permanganate is warmed to 40° with dilute hydrochloric acid, the odor of hypochlorous acid is very marked and a precipitate of manganese dioxide is formed.

temperatures with manganous salts, according to Guyard's reaction, to form a compound of trivalent or quadrivalent manganese, which, in turn, is capable of rapidly oxidizing oxalic acid.[1] Since manganous salts are produced by the reduction of the permanganate, in titrating with permanganate, in the presence of hydrochloric acid, at high temperatures the Guyard reaction is accelerated to such an extent that the concentration of the hypochlorous acid is kept at a minimum, while the effect of cooling during titration is counterbalanced by the accumulating manganous salt. Obviously the temperature *at the beginning* of the titration, while the concentration of the manganous salt is low, is the important factor. In the presence of large quantities of manganous salt, as Gooch and Peters have pointed out,[2] the Guyard reaction is so much accelerated that exact results may be obtained even at 25°.

Possibly, however, an additional effect of high temperatures is to accelerate the reaction between the hypochlorous and hydrochloric acids to form chloride, which is readily taken up by oxalic acid, or that between hypochlorous acid and manganous salts to form manganese dioxide which then oxidizes the oxalic acid.

After showing the effect of temperature upon the reaction it was interesting and desirable to determine the influence of varying other conditions. In Table II. are given the results of different dilutions:

Table II.

$H_2C_2O_4$.	HCl.	Volume.	Temperature at beginning.	$KMnO_4$ used.	Theoretical $KMnO_4$.	Error in per cent.
cc.	cc.	cc.		cc.	cc.	
25	10	50	80°±	45.78	45.74	+0.09
"	"	50	80°+	39.53	39.50	+0.08
"	"	50	80°+	39.52	39.50	+0.05
"	"	100	80°±	45.78	45.74	+0.09
"	"	100	80°+	39.46	39.50	−0.10
"	"	100	80°+	39.50	39.50	0.00
"	"	100	80°+	39.48	39.50	−0.05
"	"	300	75°	45.73	45.74	−0.02
"	"	300	58°	45.81	45.74	+0.15
"	"	300	45°	45.96	45.74	+0.48

[1] Shilow: Ber. d. chem. Ges., 36, 2735 (1903). Strabal: Z. anorg. Chem., 42, 1 (1904).

[2] *Loc. cit.*

It is evident that varying the concentration, within wide limits, has no appreciable effect, but, that as before, the temperature is an extremely important factor.

A stronger solution of potassium permanganate was found to act no differently from those first employed, thus bearing out the conclusion, previously expressed, that the concentration of the permanganate ion has a comparatively slight influence. In the starred analyses the permanganate was added slowly at first, until the reaction was well started. This method of procedure is seen to have had a beneficial effect. The temperature, below which an error is introduced in the case of the more concentrated permanganate also, is very close to 70° (Table III.):

Table III.

$H_2C_2O_4$. cc.	HCl. cc.	Volume. cc.	Temperature at beginning.	$KMnO_4$ used. cc.	Theoretical $KMnO_4$. cc.	Error in per cent.
25	10	150	80°	21.68	21.70	—0.09
"	"	"	80°	21.70	"	0.00
"	"	"	90°	21.70	"	0.00
"	"	"	90°	21.68	"	—0.09
"	"	"	75°	21.70	"	0.00
"	"	"	75°	21.70	"	0.00
"	"	"	70°	21.70	"	0.00
"	"	"	70°	21.72	"	+0.09
"	"	"	70°	21.71	"	+0.05
"	"	"	70°	21.70*	"	0.00
"	"	"	65°	21.72	"	+0.09
"	"	"	65°	21.70*	"	0.00
"	"	"	65°	21.70*	"	0.00
"	"	"	60°	21.74	"	+0.18
"	"	"	60°	21.72	"	+0.09
"	"	"	60°	21.77	"	+0.32
"	"	"	60°	21.77*	"	+0.32
"	"	"	60°	21.77*	"	+0.32
"	"	"	60°	21.74*	"	+0.18
"	"	"	60°	21.74*	"	+0.18
"	"	"	55°	21.81	"	+0.51
"	"	"	55°	21.74*	"	+0.18

Next the effect of increasing the concentration of the hydrochloric acid only was investigated and it was found that a

slight positive error, which increased with the concentration of the hydrochloric acid and diminished with the temperature, was introduced (Table IV.). In the last analysis the end-point, though sharp, soon faded :

Table IV.

$H_2C_2O_4$. cc.	HCl. cc.	Volume. cc.	Temperature at beginning.	$KMnO_4$ used. cc.	Theoretical $KMnO_4$. cc.	Error in per cent.
25	20	150	80°±	45.83	45.74	+0.20
"	20	"	85°	21.69	21.70	—0.05
"	20	"	75°	34.94	34.93	+0.03
"	20	"	85°	34.94	34.93	+0.03
"	20	"	80°	34.94	34.93	+0.03
"	30	"	80°±	45.78	45.74	+0.09
"	30	"	65°	45.80	45.74	+0.13
"	30	"	95°	21.74	21.70	+0.18
"	40	"	60°	45.86	45.74	+0.26
"	50	"	75°	45.84	45.74	+0.22
"	50	"	95°	21.73	21.70	+0.13

Finally, the concentration of the chlorine ion was varied by adding sodium chloride to the oxalic acid solution :

Table V.

$H_2C_2O_4$. cc.	HCl. cc.	Volume. cc.	Temperature at beginning.	NaCl added. Grams.	$KMnO_4$ used. cc.	$KMnO_4$ theory. cc.	Error in per cent.
25	10	150	80°	3	39.48	39.50	—0.05
"	"	"	80°	3	39.47	39.50	—0.07
"	"	"	80°	5	39.50	39.50	0.00

In order to show that no oxalic acid is either volatilized or decomposed at high temperatures, in two cases titrations, in the presence of sulphuric acid, were carried out with portions which had been heated with the sulphuric acid at 88°–90°, in an open beaker, for 15 and 30 minutes, respectively. The theoretical amount of permanganate necessary was 21.70 cc., while 21.68 cc. were used in both cases. Evidently the volatilization or decomposition of oxalic acid is negligible under ordinary conditions and the precaution of keeping the temperature of the solution below 60°, which is generally recommended in text-books, is unnecessary.

The potassium permanganate solutions which were employed

in the foregoing analyses were made by dissolving the salt in hot water and allowing the solution to stand for at least 24 hours, then decanting the clear liquid. The solutions did not come in contact with rubber or any reducing substance and their strengths were determined on the day they were used. Titrations were made by both the authors, with confirmatory results in all cases.

The main results of the research are as follows:

1. It is found that oxalic acid may be determined, with great exactness, by titration with potassium permanganate, in the presence of hydrochloric acid, if the following precautions are observed:

a. The temperature of the solution, at the beginning of the titration, must be at least as high as 70°.

b. The concentration of the hydrochloric acid must not be greater than 20 cc. of dilute acid, of sp. gr. 1.04–5, in 150 cc.

c. The solution must not be too concentrated. One hundred and fifty cc. is a suitable volume for 0.3 gram of oxalic acid.

d. The permanganate must not be added too rapidly and constant stirring is advisable.

2. A tentative explanation is proposed for the cause of the loss of chlorine from the solution and consequent use of too much permanganate when the above precautions are neglected.[1]

3. It is shown that oxalic acid does not appreciably volatilize or decompose, in dilute solution, at temperatures as high as 90°.

CAMBRIDGE, MASS.,
January 12, 1905.

REPORTS.

Krafft's Work on Molecular Weight Determinations in a Vacuum.

Professor F. Krafft, of Heidelberg, has made a prolonged study of the behavior of substances of high molecular weight in a vacuum and of the evaporation-points and boiling-points of metals in a vacuum. His first publication on the subject

[1] Further evidence upon this point will be presented in a subsequent paper upon the titration of ferrous iron with permanganate, in the presence of hydrochloric acid.

appeared in 1895. A list of subsequent articles is given below;[1] the last three articles are the most important. His studies have led him to a practical method of determining the molecular weight of high molecular substances and to interesting results concerning other constants of matter. The method is based on the following observations.

The passage of matter from the solid or liquid to the gaseous state depends on two factors: first, on overcoming the force of gravity; second, on overcoming the pressure of the atmosphere.

If, by working in a nearly perfect vacuum, the factor of atmospheric pressure is eliminated, we have to deal with gravity alone and the simple quantitative relation of substances to one another, in passing into the liquid or gaseous state in a vacuum, must be regarded as functions of molecular weight. How clearly defined these relations are is shown by this example:

Hexadecane, $CH_3(CH_2)_{14}CH_3$. Molecular weight, 226.		Dotriacontane, $CH_3(CH_2)_{30}CH_3$. Molecular weight, 450.	
Height of vapor column.	Boiling-point.	Height of vapor column.	Boiling-point.
65 mm.	64°	65 mm.	202°
175 mm.	82°	175 mm.	238°
Difference,	18°	Difference,	36°

Here we have two strictly analogous molecules, one of which has twice the weight of the other. To lift the heavier molecule to a given height requires just twice the heat energy required to lift the lighter molecule.

In this process each layer of vapor lifts up the layer above it and is itself cooled by this work; hence, in the upper layers, on which there is practically no more vapor pressure, we reach the temperature at which the force of gravity is just overcome. This temperature is the "*vacuum boiling-point.*"

With substances of high molecular weight there is no difficulty in reading the height of the vapor column within a millimeter.

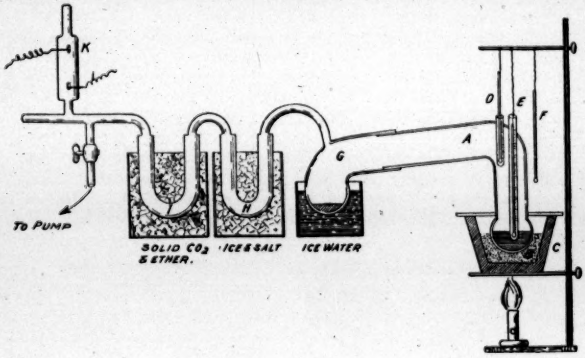
The sketch on the following page shows the apparatus used in determining molecular weight.[2]

To avoid, as far as possible, vapor-pressure or friction in the distilling-flask *A*, Krafft makes it in the form of a bent tube, of uniform diameter above the bulb and to avoid reading the

[1] Ber. d. chem. Ges., **28**, 2583; **29**, 1316, 2240; **32**, 1623; **33**, 3207, 3576; **35**, 1690, 4339, 4344; **37**, 95; **38**, 242, 254, 269.

[2] Professor Krafft gives no drawing of his apparatus. As it is a help to understanding his method, the writer has made a diagrammatic representation from the description.

height of vapor column, he makes the flask with a uniform height of 195 mm. from the surface of the liquid substance to the bend. The bulb is partly immersed in a Wood's metal-bath, in the crucible *B*, the temperature of which is kept con-



stant by an outer crucible *C*, the space between the two iron crucibles being filled with asbestos. *D* is a thin glass tube, open at the top, fused into the flask, so that the bottom comes just below the bend; *E* is a similar tube reaching nearly to the bottom of the flask; these tubes, *D* and *E*, are the containers for the thermometers, which can be lowered in the tubes to any desired point. *F* is a thermometer, to determine the temperature of the air. A broad mica plate, bored to admit the flask, covers the bath, it protects the thermometers from radiated heat. The distilling-flask is ground into the receiver *G*, which stands in ice-water and is connected with a U-tube *H*, standing in ice and salt; this is to prevent the possible escape of vapor into the pump. A second U-tube, *I*, which can be cooled at the proper moment, by a mixture of solid carbon dioxide and ether, comes next. A tube connects the apparatus with a powerful mercury pump and the cathode light, in a Crookes-Lenard tube *K*, serves as an indicator of the vacuum.

In making a determination, the substance examined is put in the bulb of flask *A*, the apparatus connected with the pump, a current from an induction coil passed through the Crooke's tube and the substance heated by means of the metal-bath. When the bright green cathode light indicates very low pres-

sure and the substance is boiling and distilling steadily, solid carbon dioxide and ether are put in the receptacle around the U-tube *I*; this lowering of temperature lessens the pressure to such an extent that the cathode light disappears; Krafft says the pressure is less than one-millionth of an atmosphere, practically a vacuum; the pressure is now a negligible factor. The thermometers are read, which finishes the determination. $M = E \times C$; M is the molecular weight sought, E is the difference in temperature indicated by the upper and lower thermometers. C is a constant of the apparatus used. It depends on the height of the vapor column, the diameter of the flask, etc. and must be determined for each distilling-flask, by a trial with some substance of known molecular weight; for example, a flask tested with lauric acid ($M = 200.0$) gave

$$C = \frac{M}{E} = \frac{200.0}{22.0} = 9.009,$$

the constant for that flask.

The results tabulated below show how close the observed differences were to those calculated:

Substance.	Molecular weight.	Difference in boiling-point found.	Difference in boiling-point calculated.
m-Dinitrobenzene	168	17°.5	18°.6
Benzidine	184	20°.0	20°.4
Phenyl benzoate	198	20°.5	21°.9
Lauric acid	200	22°.2	22°.2
Mercury	200	22°.0	22°.2
Benzil	210	23°.5	23°.3
Palmitic acid	256	28°.5	28°.4
Nonadecane	268	30°.5	29°.7
Eicosane	282	31°.5	31°.3
Cetyl iodide	352	35°.0	39°.1
Dotriacontane	450	50°.0	50°.0

Krafft published, in 1903,[1] an interesting article on the evaporation and boiling of metals in quartz flasks. In the course of it he calls attention to the observation that, in a vacuum, evaporation precedes boiling very markedly; indeed some metals volatilize slowly, in a vacuum, several hundred degrees below their boiling-points under similar conditions and form a bright, metallic mirror on the cooler wall of the apparatus. He has taken up this work again and determined, for a number of metals, the lowest temperatures at which they evaporate in a vacuum. In experiments for this purpose it was

[1] Ber. d. chem. Ges., 36, 1690.

necessary to avoid all pressure of vapor, *i. e.*, all force of gravity. The apparatus was a horizontal glass tube 1 cm. in diameter and 25 cm. long, ground into a receiver and connected with a pump and Crookes' tube; the glass tube is closed at the far end and for 8 cm distance from the closed end the tube is surrounded by an outer tube, fused on to it, resembling the inner and outer tubes of a Liebig condenser; the space between the tubes was filled with a suitable liquid and heated, to secure an even temperature, the substance being put near the closed end of the inner tube. As noted in the table below, the evaporation-points of a few metals range from —40° for mercury to 640° for silver. The experiments with silver and other high-fusing metals were made in a tube of quartz heated in a small electric furnace.

It was shown in the first pages of this report that, in boiling in a vacuum, a vapor is formed which we may call "saturated" in relation to gravity. In boiling under a pressure of 760 mm. the vapor is "saturated" in relation to gravity plus atmospheric pressure. It would be of interest to bring these two boiling-points into measurable relation and this can be done by considering the two "limit conditions" in combination with a third "limit condition," the beginning of evaporation in a vacuum.

Mercury begins to evaporate in a vacuum at —40° and boils at 155°; under atmospheric pressure it boils at 357°. The interval between the first and second temperatures is 195°; the interval between the second and third is 202°; the intervals are approximately equal; how close these relations are is shown in this table:

Element.	Beginning of evaporation in vacuum.	Boiling-point in vacuum.	Difference, I.	Boiling-point, 760 mm.	Difference, II.
Mercury	—40°	155°	195°	357°	202°
Cadmium	156°	450°	294°	749°	299°
Zinc	184°	550°	366°	920°	370°
Potassium	63°	365°	302°	667°	302°
Sodium	98°	418°	320°	742°	324°
Bismuth	270°	993°	723°	1700°	707°
Silver	680°	1360°	680°	2040°	680°

If, as shown above, the essential work during boiling in a vacuum consists in overcoming the force of gravity, then this table shows that the elements examined require the same amount of heat from the beginning of the evaporation till gravity is overcome—as is required to overcome atmospheric pressure.

To sum up, Krafft maintains that boiling in the air depends on two factors, gravity and atmospheric pressure. By comparing vacuum boiling-points and air boiling-points he has a measure of atmospheric pressure. He believes that the lowest evaporation-point is the temperature at which single molecules begin to escape from the heated substance and that the interval between the evaporation-point and the vacuum boiling-point is a measure of gravity.

If he is right, it follows that, *at the earth's surface, gravity and atmospheric pressure are equivalent.* E. RENOUF.

The Triphenylmethyl Question.

The triphenylmethyl question has assumed such an important position in the literature of organic chemistry that it has seemed well to prepare a brief report upon our knowledge of the substance. The work of Gomberg is so well known that only a few of the more characteristic properties of triphenylmethyl will be described here, while the greater part of this report will deal with certain suggestions as to the structure of triphenylmethyl which have been presented recently.

Triphenylmethyl was first obtained by Gomberg, in 1900, by the action of zinc upon triphenylchlormethane. The substance was found to be remarkably sensitive to certain reagents. It unites with atmospheric oxygen to form a peroxide, absorbs halogens readily and, in every way, exhibits the characteristics of an unsaturated compound. Finally, the cryoscopic determinations of the molecular weight, first made by Gomberg, indicated the unsaturated formula of triphenylmethyl as the correct expression of the structure of the substance. Later, however, Gomberg repeated his molecular weight determinations and found that his second series of measurements pointed to the double formula.[1] Notwithstanding this fact, the formula of hexaphenylethane could not be ascribed to Gomberg's substance for the reason that Ullmann and Borsum[2] had obtained a hydrocarbon, by another method, to which they had given this formula. This substance, too, had previously been obtained by Gomberg,[3] by a still different process. Since the so-called hexaphenylethane of Ullmann and Borsum was not identical with the triphenylmethyl of Gomberg, it became necessary to look elsewhere for an explanation of the phenomenon.

In a recent number of the *Berichte*,[4] A. E. Tschitschibabin

[1] Ber. d. chem. Ges., **37**, 2037 (1904).
[2] *Ibid.*, **35**, 2877 (1902).
[3] *Ibid.*, **35**, 3914 (1902).
[4] *Ibid.*, **37**, 4712 (1904).

points out that the hydrocarbon of Ullmann and Borsum is not hexaphenylethane, but an isomeric substance and that, in all probability, triphenylmethyl is the true hexaphenylethane. Tschitschibabin's argument is as follows:

The hydrocarbon, obtained by Ullmann and Borsum and considered by them to be hexaphenylethane, was prepared by the action of zinc upon triphenylchlormethane in glacial acetic acid solution. In order to bring about a reaction, however, it was necessary to add zinc chloride. In their paper Ullmann and Borsum[1] express the belief that the reaction takes place in the following stages: Triphenylchlormethane is hydrolyzed by the small amount of water present in the acetic acid used and the triphenylcarbinol thus formed is reduced to hexaphenylethane by the hydrogen resulting from the action of the zinc upon the acetic acid:

$$2(C_6H_5)_3C.OH + 2H = (C_6H_5)_3C—C(C_6H_5)_3 + 2H_2O.$$

Since a condensing agent is necessary to bring about the reaction, Tschitschibabin thinks it probable that the condensation takes place in another manner and that pentaphenyldimethylbenzene (diphenylomethyltetraphenylmethane) is produced:

$$(C_6H_5)_3C.OH + C_6H_5.CH(C_6H_5)_2 = (C_6H_5)_3C.C_6H_4.CH.(C_6H_5)_2 + H_2O,$$

or that the condensation may take place between 2 molecules of the carbinol with a subsequent reduction to the above hydrocarbon. This reaction is made probable by the work of Baeyer and Villiger,[2] who showed that triphenylcarbinol condenses with phenol in an analogous manner:

$$(C_6H_5)_3C.OH + C_6H_5OH = (C_6H_5)_3C.C_6H_4OH + H_2O,$$

and also by the work of Ullmann,[3] who represented the condensation between triphenylcarbinol and anilin by the equation:

$$(C_6H_5)_3C.OH + C_6H_5NH_2 = (C_6H_5)_3C.C_6H_4NH_2(p) + H_2O.$$

Ullmann and Borsum studied their substance carefully and came to the conclusion that its structure must be represented by the formula of hexaphenylethane. Tschitschibabin found, however, that, if dissolved in carbon bisulphide and placed in the sunlight, the hydrocarbon took up 1 atom of bromine and liberated hydrobromic acid. From this bromine derivative he was able to pass to the carbinol, acetyl and ethoxy compound. Finally, he proved that the bromine derivative is not a mixture

[1] Ber. d. chem. Ges., **35**, 2877 (1902).
[2] *Ibid.*, **35**, 3018 (1902).
[3] *Ibid.*, **36**, 404 (1903).

of substances containing bromine in a benzene nucleus. These reactions point, unmistakably, to the presence of a methane hydrogen atom and must be represented as follows:

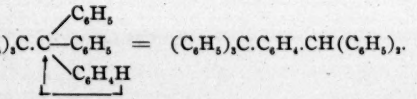
$$(C_6H_5)_3C.C_6H_4CH(C_6H_5)_2 + Br_2 = (C_6H_5)_3C.C_6H_4.CBr(C_6H_5)_2 + HBr,$$

on treatment with water

$$(C_6H_5)_3C.C_6H_4.CBr(C_6H_5)_2 + H_2O = (C_6H_5)_3C.C_6H_4.C(OH)(C_6H_5)_2 + HBr.$$

From the results of these experiments Tschitschibabin suggests, as already mentioned, that triphenylmethyl is the true hexaphenylethane. He thinks that the marked reactivity of triphenylmethyl is not inconsistent with the structure of hexaphenylethane, because the presence of many electronegative groups tends to increase the activity of compounds. Peculiarities of structure often cause saturated compounds to act as though unsaturated; *e. g.*, trimethylene passes readily into trimethylene bromide. Again, the work of Engler, Weger[1] and others has shown that many substances react with the oxygen of the air, at ordinary temperatures, to form peroxides as their first product. This phenomenon occurs with certain saturated compounds and is, probably, more common than is ordinarily supposed. Finally and most important, Gomberg has shown that triphenyliodomethane forms a peroxide with the same ease as triphenylmethyl itself.

In the succeeding number of the *Berichte* an article by P. Jacobson appears,[2] in which he offers another suggestion. The fact, shown by Gomberg, that triphenylmethyl is converted, by hydrochloric acid, into the compound of Ullmann and Borsum, makes it seem improbable that triphenylmethyl is the true hexaphenylethane, especially when the structure assigned to the Ullmann and Borsum compound by Tschitschibabin is considered. If so, this rearrangement must take place in the following manner:

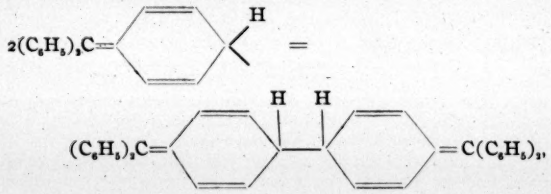


While analogy for such a transposition exists in the semidine rearrangement of the hydrazo bodies,

[1] Ber. d. chem. Ges., **36**, 309 (1903).
[2] *Ibid.*, **38**, 196 (1905).

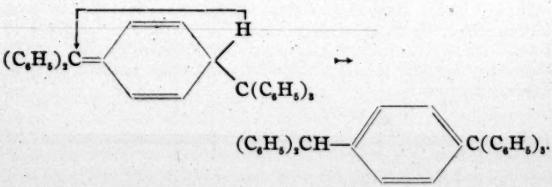
it seems improbable that it should take place in this case.

Jacobson's suggestion is founded upon the formula of a di-quinoid proposed by Heintschel :[1]



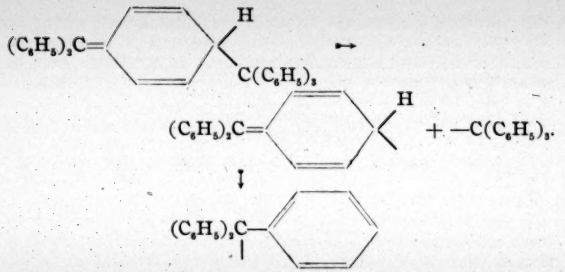
but with the difference that one quinoidal triphenylmethyl group is combined with one non-quinoidal triphenylmethyl complex, thus :

It has been shown by the work of Auwers, Bamberger, Zincke and others, that these quinoidal compounds exhibit a marked tendency to return to the aromatic condition. Should such a substance as the above rearrange, by the shifting of a hydrogen atom, the hydrocarbon of Ullmann and Borsum, as described by Tschitschibabin, would be formed :



On the other hand, should the aromatic triphenylmethyl residue detach itself, a quinoidal triphenylmethyl would remain which could easily rearrange itself into the aromatic form by the shifting of its bonds :

[1] Ber. d. chem. Ges., **36**, 320, 579 (1903).



Thus such a substance could readily react as triphenylmethyl and would be in accord with Gomberg's latest molecular weight determinations.

In a later number of the same journal[1] Tschitschibabin discusses the suggestion of Jacobson. He admits that the change of triphenylmethyl into the hydrocarbon of Ullmann and Borsum is easily explained by the quinoidal formula, but sees no special advantage in the monoquinoidal formula of Jacobson over the diquinoidal formula of Heintschel. Both quinoidal formulas seem improbable, however, when certain facts are considered. There undoubtedly exists a close relationship between triphenylmethyl and its haloid compounds. This is shown by the easy formation of triphenylmethyl by the action of metals; such as silver, on the haloid compounds. Since the work of Baeyer and Villiger makes it improbable that these haloid compounds have the quinoidal structure, it is hardly probable that triphenylmethyl possesses it. There seems to be no reason to doubt the capability of existence of such compounds as hexaphenylethane and, if Gomberg's hydrocarbon is indeed such, the change into the hydrocarbon of Ullmann and Borsum, by means of hydrochloric acid, can readily be explained by the assumption that the elements of hydrochloric acid are absorbed, with the formation of 1 molecule of triphenylmethane and 1 of triphenylchlormethane and that a condensation between these then occurs, with the liberation of hydrochloric acid:

$$(C_6H_5)_3C.C(C_6H_5)_3 + HCl = (C_6H_5)_3CH + (C_6H_5)_3C.Cl;$$

$$(C_6H_5)_3C.Cl + C_6H_5CH(C_6H_5)_2 = (C_6H_5)_3C.C_6H_4.CH(C_6H_5)_2 + HCl.$$

The question is still an open one. Both Tschitschibabin

[1] Ber. d. chem. Ges., **38**, 771 (1905).

and Jacobson's ideas are valuable, whether or not either of them has reached the truth. The next paper of Gomberg, in which he will doubtless consider these suggestions, will be awaited with interest. P. H. Cobb.

REVIEWS.

THE STUDY OF THE ATOM; OR THE FOUNDATIONS OF CHEMISTRY. By F. P. VENABLE, PH.D., D.SC., LL.D., University of North Carolina. Easton, Pa.: The Chemical Publishing Company. 1904. pp. 290.

In the words of the author, "The purpose of this work is to trace the atomic theory of chemistry from its earliest conception to the present day. This forms the foundation of all chemical theory and has been offered as the best explanation of the constitution of matter and of the universe. This theory has had a longer life than any other philosophical or scientific conception, and has to-day more nearly its ancient form. It has lived through bitter attack, dialectic strife, and even persecution, and can number its martyrs. It has called to its service the master minds of the world and the greatest ingenuity in experiment and logic. It is not to be presumed that such a conception can be dismissed in a few slighting sentences or overturned by one or two crude hypotheses."

In successive chapters the author takes up:

I. Ancient views as to the nature of matter.
II. From the Greek philosophers to Dalton.
III. The atomic theory of chemistry.
IV. The relative weights of the atoms.
V. The periodic or natural system.
VI. Affinity, the atomic binding force.
VII. Valence.
VIII. Molecules and the constitution of matter.

He clearly recognizes that the early atomic theories were of no value to chemistry. They were not chemical theories. The title of Chapter III. emphasizes this—"The atomic theory of chemistry," which is, of course, the theory of Dalton. An unusually clear account of the evolution of Dalton's views is given, use being made of the recent work of Roscoe and Hardin.

The author has not, to say the least, lost his head on the subject of ions. In speaking of the ionic theory he says (p. 215): "There are many difficulties in the way of this theory and in some respects it is not a satisfactory solution of the problems. It is still under discussion. Manifestly it is of very limited application whatever of truth there may be in it, since

it applies fully only to certain bodies dissolved in particular solvents and then only in very dilute solutions." It may be said that the fact that substances are not, as a rule, completely dissociated is not to be regarded as an objection to the theory. It seems highly probable that dissociation of some kind is a factor of importance in a large proportion of the reactions that take place between substances. The theory has no doubt been, and still is, a powerful incentive to valuable experimental work.

Nothing new is presented under the head of Valence, that ever puzzling subject. The hypothesis of Richards, and that of Venable, are given some space. The latter no doubt tends in the right direction, though somewhat vague.

In the last chapter, which deals with "Molecules and the Constitution of Matter," all the modern improvements including electrons and the ether of Mendeléeff are met with and any one reading this chapter will have a very fair idea of the views that are now under discussion. In fact the whole book is recommended to those interested in the theories of chemistry.

Some errors have been noted. On page 43 "Berthollet" is used three times for "Berthelot." On page 83 the title of Berthollet's book should be "Essai d'une Statique chimique." On page 84 "Stoichiometrie" should be "Stöchiometrie." On page 122 "Ampere should be "Ampère." On page 199 the title of Berthollet's book is again wrong, being given as "Essai de statique chimique." These are, however, small matters, and can be easily corrected. I. R.

AN ATTEMPT TOWARDS A CHEMICAL CONCEPTION OF THE ETHER. By PROFESSOR D. MENDELÉEFF. Translated from the Russian by GEORGE KAMENSKY, A.R.S.M., of the Imperial Mint, St. Petersburg. London, New York and Bombay: Longmans, Green & Co. 8vo. 1904. pp. 51. Price, 80 cents.

The author is of the opinion that the ether must be a definite chemical substance and must have weight. He says, "Although sceptical indifference is prone to discern only a working hypothesis in the conception of the ether, yet the earnest investigator, seeking the reality of truth and not the image of fantasy, is forced to ask himself what is the chemical nature of the ether." * * * "If the question were restricted to the ether which fills space and serves as a medium for the transmission of energy, it would in a way be possible to limit oneself to the supposition of mass without reference to its chemical relations and even to consider the ether as a primary matter, just as the mass of a planet may be conceived without regarding its chemical composition. But such an indifferent, in-

definite ether loses all sense of reality and awakens the misgivings of the earnest investigator, directly he realizes that it must permeate all substances."

The investigation into the chemical nature of the ether is based upon two propositions: "(1) That the ether is the lightest (in this respect ultimate) gas and is endowed with a high penetrating power, which signifies that its particles have, relatively to other gases, small weight and extremely high velocity, and (2) that the ether is a simple body (element) incapable of entering into combination or reaction with other elements or compounds, although capable of penetrating their substance, just as helium, argon, and their analogues are soluble in water and other liquids."

An extension of the periodic arrangement of the elements provides a place for an element x in the zero group, which includes helium, neon, argon, krypton, and xenon, and series 0 which precedes series 1. In series 1 the only known element included is hydrogen. The author now assigns an unknown element y to this series. This element is the second member of the zero group. The atomic weight of y is not greater than 0.4 and is probably less. "Such an analogue of helium may perhaps be found in coronium, whose spectrum, clearly visible in the solar corona above (that is, further from the sun than) that of hydrogen, is simple like that of helium, which seems to indicate that it belongs to a gas resembling helium, which was also predicted from its spectrum by Lockyer."

But y with an atomic weight 0.4 and a probable density of 0.2 "cannot possibly be ether, its density being too great. It wanders, perhaps for ages, in the regions of space, breaks from the shackles of the earth and again comes within its sphere, but still it cannot escape from the region of the sun's attraction, and there are many heavenly bodies of greater mass than the sun. But the atoms of ether must be of another kind; they must be capable of overcoming even the sun's attraction, of freely permeating all space, and of penetrating everything and everywhere. The element y, however, is necessary for us to be able to mentally realize the lightest and, therefore, swiftest element x, which I consider may be looked upon as the ether." It is evident that the atomic weight of x must be so small that the element will not be held in the atmosphere of any of the stars. By means of a few assumptions which seem justifiable and a few simple calculations the author reaches the conclusion that "*the particles and atoms of the lightest element x, capable of moving freely everywhere throughout the universe have an*

atomic weight nearly one-millionth that of hydrogen and travel with a velocity of about 2250 kilometers per second."

All chemists and physicists will find this pamphlet interesting and suggestive. I. R.

GRUNDZÜGE DER CHEMISCHEN PFLANZENUNTERSUCHUNG. By DR. L. ROSENTHALER. Berlin: Julius Springer. 1904 Price, 2.40 M.

This little book of 124 pages is intended to help the beginner, who seeks to know the real composition of plants, to proceed in a systematic way with his investigation.

In the introduction the author calls attention to the fact that it cannot be expected, in a complex like a plant, to work with the accuracy and certainty which characterize the methods applied to inorganic mixtures and even the best quantitative processes give, finally, only approximate results. Often it is far easier to be sure of the absence of a group of substances than of their presence. For example, if, in a plant extract, the common reagents which precipitate alkaloids give no reaction we may know that alkaloids are absent, but if a precipitate is produced it may be due to other nitrogenous bodies. The needs of plant chemistry are greater than ever, more is demanded by the physiologist, the pharmacist and the pharmacologist and the importance of the subject is such as to cause the author to demand that a thorough and systematic study of it be made in the universities.

The general part of the book treats of the methods of preparing the material—powdering, percolating, extracting, etc.

The preliminary tests to determine the character of the constituents are next described. The process of Stas-Otto for the solution of the glucosides and bitter principles and the separations effected by lead acetate are described in sufficient detail.

The special part treats of the separation and determination of the alkaloids, glucosides, fats, and oils, waxes, lecithins, ethereal oils, resins, tannins, phlotophenes, organic acids, albuminoids and their decomposition-products, enzymes, toxalbumins, carbohydrates and related substances and inorganic compounds.

Of particular interest are the data relating to enzymes and toxalbumins because the older works on this subject do not discuss these points.

The manual cannot fail to be of use both to the student and the chemist, engaged either in analysis or research.

H. W. WILEY.

ELEKTROLYTISCHE VERFAHREN ZUR HERSTELLUNG PARABOLISCHER SPIEGEL. Von SHERARD COWPER-COLES, London. Zur Deutsche übertragen von DR. EMIL ABEL. Mit 13 Figuren und 2 Tabellen im Text. Verlag von Wilhelm Knapp, Halle, a. S. 1904. pp. 17. Price, M 1.00.

This little monograph contains a clear and concise account of the processes involved in the preparation of parabolic mirrors. H. C. J.

MONOGRAPHIEN ÜBER ANGEWANDTE ELEKTROCHEMIE. XV. Band. Künstlicher Graphit. Von FRANCIS A. J. FITZ-GERALD, Chemiker der International Graphite Co., Niagara Falls, N. Y. Ins Deutsche übertragen von DR. MAX HUTH, Chemiker der Siemens und Halske, A. G., Wien. Halle, a. S.: Wilhelm Knapp. 1904. Price, M. 3.

Reviews of several of these monographs have appeared in THIS JOURNAL; this one is of interest to chemists.

Mr. Fitz-Gerald first gives a brief historical sketch of the work of Despretz, Berthelot and Moissan and next considers the different methods of making graphite. Castner's patent was based on the observation that carbon electrodes, connected with a dynamo and heated by passing a powerful current through them, are converted into denser carbon, of graphitic character, losing volatile material.

Girard and Street patented a furnace for making electrodes. Carbon electrodes are passed, longitudinally, through a heated space, in which they are brought into thorough contact with the arc, converting the carbon into graphite; by shifting a magnetic field the arc is directed as desired, thus securing thorough contact aud complete change to graphite. The patentees found that an electrode made of retort carbon and tar was not as completely changed to graphite as one containing 2 per cent of silica, or boric acid, or iron oxide.

Acheson's process developed from the observation that carborundum, at high temperatures, changed to graphite, silicon being volatilized, $CSi = C + Si$. The graphite thus formed is remarkably pure, containing only 0.03 per cent impurity. The yield, however, is small; the theoretical yield would be only 30 per cent of the original material; in practice much less is obtained.

Acheson's theory is that the carbon of an impure coal unites at a high temperature with silicon, or other elements, forming pure carbides, which, at higher temperatures, dissociate into pure graphite and the other element or elements, which are volatilized. He uses either silica, alumina, clay, lime, or iron oxide in forming such carbides.

Most of the artificial graphite marketed is made from anthracite. In the patent for this process Acheson enlarges his

theory. He says that it is not necessary to add the amount of silica, or other foreign substance, required to combine with all of the carbon to form a carbide, but that a small amount of such substance can decompose large quantities of carbon, yielding graphite, the process being the formation and decomposition of carbide, into graphite and the foreign element, which unites with the carbon from anthracite again, until the carbon is thus completely changed to graphite. He finds, also, that anthracite containing, as natural impurity, 5 per cent of silica, alumina and iron oxide gives better results than any artificial mixture of these substances with pure carbon.

In a brief supplement the author refers to recent work of himself and others on this subject. E. R.

LA STATIQUE CHIMIQUE, Basé sur les Deux Principes Fundamentaux de la Thermodynamique, par le LIEUTENANT-COLONEL E. ARIES, Ancien Élève de l'École Polytechnique Librarie Scientifique. Paris: A. Hermann. 1904. pp. 251. Price, 10 Fr.

This book on chemical statics, based on the two fundamental laws of thermodynamics, is clearly and concisely written and a useful addition to the literature of the field. An idea of the scope of the book can be obtained best by a glance at its contents. It treats of mixtures, the laws and equations of chemical equilibrium, the phase rule, some types of dissociation, solutions, separation of liquid mixtures, the mixed vapor emitted by a liquid mixture, a perfect gas, the law of Dalton and the principle of Gibbs, homogeneous systems, dilute solutions and osmosis.

The book would be more easily accessible and its value would be increased by a good subject and author index.

H. C. J.

APPLICATION OF SOME GENERAL REACTIONS TO INVESTIGATIONS IN ORGANIC CHEMISTRY. By DR. LASSAR-COHN, Professor of Organic Chemistry at the University of Königsberg. Authorized translation by J. BISHOP TINGLE, PH.D. (Munich), F.C.S., Johns Hopkins University. New York: J. Wiley & Sons; London: Chapman & Hall, Limited. 1904. pp. 101.

This valuable little book should be in the hands of every one engaged in research in the field of organic chemistry. The object of the author is stated by him thus: "The time has arrived when it is possible to formulate, for the conduct of some operations, a number of general rules derived from the enormous mass of experimental material which has accumulated, and, as a corollary, to develop considerably many methods which are in use for organic chemical investigations. It may be hoped that, in course of time, the blind, trial and failure

process may be increasingly restricted; this little book is offered as an attempt to systematize methods of work and treats of the following topics: *Fixation of labile hydrogen atoms; modification of reactions; improvement in the conditions of reaction by due recognition of the circumstances in which the product is formed; influence of neighboring atoms and atomic complexes; also some remarks on the overstrain of reactions.*

Under the head of "influence of neighboring atoms and atomic complexes" the author says: "At present the number of definitely ascertained instances of protective action is somewhat limited; reactions inhibited from this cause have usually failed to be recorded on account of their negative results. It is well known that certain acids are not esterified by means of a mixture of alcohols and sulphuric acid or hydrochloric acid, and that certain nitriles and amines are not hydrolyzed by the action of dilute acids."

The writer of this notice has been much interested in phenomena of the kind here referred to and, with a number of his students, has studied many such experimentally and the results have been recorded. Apparently the articles embodying these results are unknown to the author of the book.

The translation is smooth and readable. I. R.